THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XVII JANUARY, 1941 NUMBER 1

MESODERMAL MIXED TUMORS OF THE BODY OF THE UTERUS *

AVERILL A. LIEBOW, M.D., and ROBERT TENNANT, M.D.

(From the Laboratories of Pathology of the Yale University School of Medicine, New Haven, Conn., and of the Meriden Hospital, Meriden, Conn.)

A new summary of anatomical and clinical data concerning mixed tumors arising in the body of the uterus is presented, together with experimental evidence concerning the histogenesis of these neoplasms obtained by the use of the tissue cultures. This comprises the first part of the paper. Three instances observed in this laboratory are related in the second part, and in the appendix appear brief summaries of the pertinent features of previously reported cases.

PART I

The diagnosis of malignant mixed tumor of the uterus is based upon the demonstration of unusual tissues as cartilage, bone or striated muscle. Other uterine neoplasms composed of malignant mesodermal elements include the leiomyosarcoma, endometrial sarcoma, carcinosarcoma and teratoma.

The status of the carcinosarcoma is uncertain. By this term is usually implied a tumor in which spindle-shaped elements are intimately intermingled with more or less typical epithelial cells and not one resulting from collision of a separate carcinoma and

*Presented, in part, at the Fortieth Annual Meeting of the American Association of Pathologists and Bacteriologists, Pittsburgh, Pa., March 21, 1940.

Supported by a grant from the Fluid Research Funds of the Yale University School of Medicine.

Received for publication July 8, 1940.

a sarcoma. These have been discussed in general by Claessen and Mathias and have been described in the uterus by Saltykow and by Manheims, among others. It is exceedingly difficult in many instances to state whether both stroma and epithelium have assumed malignant properties or whether the epithelial cells have merely become spindle shaped. In work with the transplantable tumors of mice, it has often been observed that an epithelial neoplasm may, with successive transplantations, come to resemble a sarcoma. Such for example was the fate of the tumor of the mouse now known as Sarcoma 37 and the same transformation may occur with pulmonary carcinoma of mice induced with dibenzanthracene (Andervont). An epithelial origin of such specialized mesodermal tissues as cartilage and striated muscle, however, is far more difficult to envision.

True teratomas of the uterus, containing derivatives of all three germ layers, have been reported only a few times; by Mann, by Hellendall, and by Lackner and Krohn.

Incidence

The authors have been able to find only 65 previously recorded instances of mixed tumors of the body of the uterus. Piquand discovered cartilage in 2 of 151 sarcomas of the body of the uterus. Among malignant neoplasms of the uterus sarcomas are variously stated to occur in a proportion of 1:40 (Bunten), 20:1000 (Mathias) and 46:1082 (Frankl). These differences probably depend upon the interpretation of the possible malignancy of tumors composed of smooth muscle. Most writers state that mixed tumors of the cervix are more common than those of the body, but Meikle is in disagreement. It is interesting that mixed cervical tumors were described earlier than those of the body of the uterus and that at the time of Wilms's monograph the former were already well known whereas the latter received no mention. If one accepts the figures of Piquand and Mathias, 1 in every 7,500 malignant tumors of the body of the uterus is of this variety. It is probable that many instances have been missed because of the difficulty of demonstrating striations in the muscle fibers.

TABLE I
Modes and Sites of Origin

		Diffuse: obliterating uterine cavity	Arising diffusely as papillary or botryoid masses	Localized origin Pedunculated or sessile masses			Exact manner of origin not stated
				Posterior wall	Cornu and lateral wall	Anterior wall	
Containing striated muscle	Fundus		Gamper	Frankl (case 3) Glynn and Bell (case 1) Glynn and Bell (case 2) Gunning and Ross Halter von Franqué	Amolsch Herb Reeb and Oberling	Hunziker Lochrane	Bystroumow and Eckert Frank Liebow and Tennant (case 3) Shapiro
	Cervix and fundus		Läwen				Anderson and Edmansson Colomiatti Robertson
Containing cartilage and other tissues	Fundus	Hartfall (case 2) Jessup Kleine Sophian (case 2) van Akkeren	Frankl (case 1) Köhler Murray and Littler Perlstein Wiener	Azzola Chavannaz and Nadal (case 1) Chavannaz and Nadal (case 2) Hartfall (case 3) Liebow and Tennant (case 1) Liebow and Tennant (case 2) Olinder Rankin and Broders (case 1)	Gaebelein Gebhard Geisler Hartfall (case 1) Hofbauer Kistler Lahm Reinecke	Fels Penkert	Blasek (case 1) Blasek (case 2) Delagenière and Beauchef Frankl (case 2) Kaufmann (case 1) Kaufmann (case 2) McDonald, Broders and Counseller Nicholson Petersen (case 1) Petersen (case 2) Reid Schröder and Hillejahn Sophian (case 1) Stout Wagner Wolfe
	Cervix and fundus						Durante and Roulland Malapert and Morichau-Beauchant Seydel

Pathology

In discussing all available material it is necessary to distinguish such tumors as arise solely from the fundus from those involving also the cervix until it can be established whether or not they are fundamentally different. Furthermore, it may be well to make a grouping depending on manner of growth and site of attachment. As to the former it must be considered whether the neoplasm arose diffusely, obliterating the uterine cavity; whether it was multicentric, having a papillary or botryoid character; or whether it was polypoid. These groups in turn may be subdivided on the basis of the nature of the constituent cells. It seems reasonable to assume, as will be indicated, that tumors containing striated muscle are least likely to result from metaplasia of preëxisting elements and therefore demand some other explanation. Next in order of probability of nonmetaplastic origin are the tumors composed of several tissues, some heterotopic.

An analysis of Table I, which was constructed with these principles in mind, reveals that most of these tumors are polypoid and attached either in the region of the cervix or upon the posterior wall of the fundus. They often traverse the cervical canal and enter and even distend the vagina as partially necrotic and hemorrhagic masses yielding a sanguineous, serosanguineous or foul seropurulent discharge. Relatively few arise as multiple papillae or diffusely.

Varieties of Tissue

The types of tissue contained are indicated in Table II. Subdivision is made depending on whether striated muscle or cartilage was the predominant constituent. Most of the tumors contained cartilage and undifferentiated elements with or without other tissues. It is interesting to note that only four in the group included both cartilage and striated muscle.* Tumors consisting chiefly of striated muscle were more rarely mixed with other tissues than were those composed chiefly of cartilage. In both groups epithelium of various types was the most frequent additional tissue. In some instances the epithelium did not have the characteristics of malignancy, but in others there was carcinoma, either glandular

* Hunziker, Gamper, Frankl and Amolsch.

or undifferentiated. Bone or osteoid tissue and smooth muscle came next in frequency. Nervous tissue was identified only twice, by Schröder and Hillejahn, and by Kleine.

TABLE II
Distribution of Tissues in Types of Tumors

Varieties of tissue	Grouping		Total
	Tumors composed chiefly of striated muscle	Tumors composed chiefly of cartilage	
Striated muscle	20	...	20
Cartilage	4	42	46
Myxosarcoma or undifferentiated sarcoma	12	36	48
Giant cells	6	7	13
Epithelium	7	19	26
Smooth muscle	2	5	7
Nervous tissue	...	2	2
Fat	...	3	3
Osteoid or bone	2	3	5
Endothelium	...	2	2

Recurrences and Metastases

Of 68 cases (including the 3 described in this paper) the fate of only 28 patients is reported. One patient (Hartfall, case 3) was in good health 5 years after operation. Another (Petersen, case 2) who had a tumor consisting largely of fat, was well 2 years after hysterectomy and 6 years after onset of symptoms. Kleine's patient was apparently well 2 years postoperatively and Gamper's, 4 years. Five others, examined clinically from 3 months to 1 year after operation, seemed free of recurrence (Halter; Liebow and Tennant, case 3; Kistler; Sophian; and McDonald, Broders and Counseller). The other 19 patients all succumbed. Four died in the period immediately after operation. Three of the 19 showed no metastases at the time of operation and 1 was without metastases when examined at necropsy. The remaining 16 all died with metastases but necropsies were available only on 4. All of these 4 * had metastases to the lungs or pleura. It is probable that metastases to the thoracic viscera were much more frequent than was apparent merely from the clinical reëxamination of the patients, especially when roentgenograms were not available. Considering all of the 19 deaths, local recurrences were most commonly found (15 of 19), sometimes with further extension into

* van Akkeren, Hartfall (cases 1 and 2) and Wagner.

adjacent peritoneum resulting in compression of the intestines or veins. One patient, reported by Delagenière and Beauchef, had a tumor of the tibia. When the extremity was amputated, the tumor was found to have the structure of a sarcoma. It was not certain whether this was a metastasis.

Metastases were of three varieties:

1. The tissues of the primary lesion and metastases were the same.
   a. Hartfall (case 1): Myxomatous tissue and cartilage
   b. Hartfall (case 2): Myxomatous tissue and cartilage
   c. Wagner: Cartilage
2. Only some of many varieties of tissue were found in the metastases.
   a. Hunziker: *Primary:* striated muscle, cartilage, round and spindle cell stroma
      Metastases: striated muscle
   b. Fels: *Primary:* sarcoma, cartilage, epithelium
      Metastases: epithelium
   c. Liebow and Tennant (case 2): *Primary:* epithelium, sarcoma, cartilage
      Metastases: myxosarcoma, epithelium
3. Only dedifferentiated tissue was found in the metastases.
   a. van Akkeren: *Primary:* cartilage
      Metastases: giant cells
   b. Glynn and Bell (case 2): *Primary:* striated muscle
      Metastases: myxosarcoma

Pathogenesis

The origin of malignant tumors composed of heterologous tissues has been the subject of much speculation.

There are some who consider them to be malignant growths of metaplastic origin. Cartilage and bone are indeed sometimes the result of metaplasia. The former may develop in myxomatous connective tissue; the latter is often found where cartilage has been before as in the bronchial rings or in dense connective tissue as, on occasion, in the substance of uterine fibroids. Pierson has found that cartilage may develop in the stroma of the rabbit's

uterus under prolonged estrinization. It is more difficult to explain the presence of striated muscle on the basis of metaplasia. More specifically, there is no evidence that striated muscle can develop from smooth muscle. In the phrase of Wilms, "striated muscle has the same relation to smooth muscle as it does to cartilage or fat."

The possibility that malignant tumors may arise from the rare benign neoplasms of heterologous tissue (see Ascher, Kworostansky, Feuchtwanger, Pietzold and others) has been discussed. Again the origin of these remains obscure. Simple heterotopic inclusions not in the form of tumors are exceedingly infrequent. Thus Meyer (1930) reported the presence of a nodule of bone in a fetal uterus and of normal cartilage in the genitalia in extrauterine position. He considered these to be the result of development of fetal inclusions, however, rather than of metaplasia. Striated muscle was found in a postpartum uterus by Nehrkorn and by Girode. Blasek has described a nodule of typical cartilage in the stroma of a fragment of otherwise normal endometrium.

The multiplicity of tissues in so many of the malignant heterologous tumors makes the theory of their development from pluripotential anlagen seem relatively attractive. A true teratoma with organized arrangement of tissues from all three germ layers has been found only a few times. The source tissue is thus multipotential rather than totipotential. In his second monograph (1900) Wilms adduced evidence in support of a cell-rest theory of origin of tumors of the lower genito-urinary tract as he had done previously for the mixed tumors of the kidney (1899). Mixed tumors of the vagina, cervix, vas deferens and bladder were discussed but not those of the body of the uterus. Inclusion of myotome and sclerotome or of the predecessors of these from the dorsal segments of the posterior portion of the body was held responsible for mixed tumors of the lower generative tract. Among others, Seydel and Meyer (1903) amplified the theory by suggesting the possibility of displacement of anlagen in various stages of differentiation during the growth of the Wolffian duct or its derivatives. Seydel presented a diagram to illustrate how a portion of the blastema might come to lie ventral to the Wolffian duct and thus be displaced. More recently Masson, who restudied the embryonal adenosarcomas of the kidney, has demonstrated

an abundance of nervous tissue within them and has indicated the probability of their derivation from primitive neuro-epithelium. This neuro-epithelium may have as its derivatives nervous tissue, "mesectodermic" elements, certain muscles and the nephrogenic mesenchyme. The capacity of the neural crests to give rise to connective tissues had previously been demonstrated by Stone who called these tissues "mesectoderm." What rôle neuro-epithelium plays in the origin of mixed tumors of the uterus remains obscure. Certainly the content of nervous derivatives in our first case is exceedingly small. Whatever the exact genesis of the tumors, the idea of pluripotential anlagen makes superfluous the once current nosological division into subgroups depending on morphology.

Reticulum stains performed on sections from case 2 of our series demonstrate the disposition of argyrophil fibers about the various constituents of the mixed tumor in a manner characteristic of epithelium, cartilage and sarcoma respectively (Figs. 11 and 12). The relation to the cartilage was as in material similarly stained from a case of chondrosarcoma of bone, *i.e.*, the fibers either ran parallel to the cartilaginous mass or seemed to enter at an angle to become lost in the hyaline matrix.

An experimental approach was available in studying the first of our tumors. This consisted of explanting the tissue into culture media in Carrel flasks. Fragments of firm, translucent tumor tissue obtained under aseptic precautions through a seared surface resulted in a growth of cells closely resembling that of embryonic human cartilage (Figs. 5–8). In both instances, cells grew from the first explants in columns which did not branch immediately. Daughter cells within any column tended to remain adjacent, trellised upon one another and intertwined, rather than to branch out at an angle. In this respect they differed from cultures of fibroblasts. Distally the cells became shorter and shorter and the terminal cell was a blunt, rounded element with a flame-shaped, free border. The tumor cells were larger than those of normal fetal cartilage. Coarser and more abundant vacuoles encircled the nucleus, which had a prominent membrane and nucleoli. Whatever the origin of these tumor cells, (whether metaplastic or dysembryogenic), they had *in vitro* many characters in common with normal cells of fetal cartilage.

Clinical Notes

The sharp difference in age incidence between mixed tumors of the vagina, of the cervix and of the body of uterus has been discussed by many authors and has been illustrated graphically by

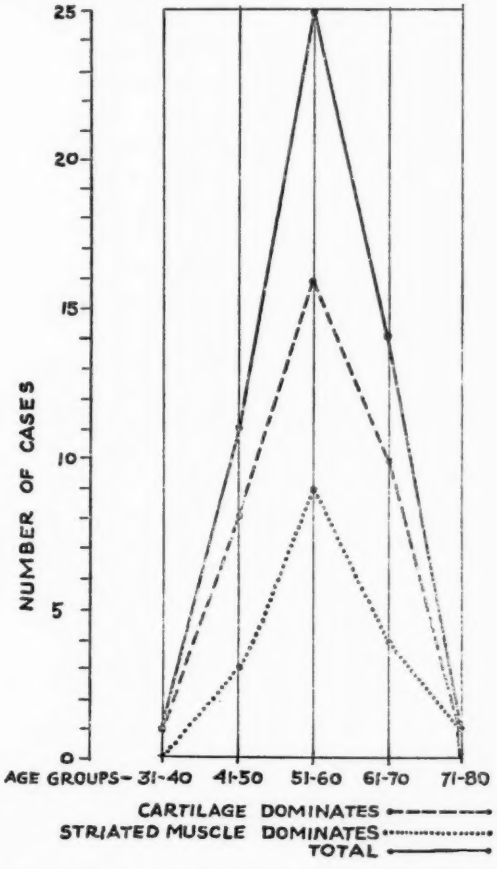


Chart 1. Distribution of all cases, of those in which cartilage dominates and of those in which striated muscle dominates, according to age groups.

Meikle. The curve obtained by plotting the data from the larger material relative to mixed tumors of the uterine body as surveyed here is presented in Chart 1. The sharp peak of the curve at

about 56 years of age is striking. Twenty per cent of the patients had never borne children.

Clinically, these tumors almost always manifest themselves by a sanguineous discharge from the vagina. Only 6 of 47 patients did not have this symptom. Five had as their chief complaint pain in the lower abdomen. Eleven others had this as a major complaint. This is noteworthy since pain is a much less common symptom with carcinoma of the fundus. The pain was described as aching, cramplike or, in 1 patient, like that in labor. In 2 others it was combined with dysuria. Perhaps pain is associated with the bulky, polypoid nature of many of these tumors about which the musculature of the uterus may contract. Two patients were conscious of an abdominal mass and 1 complained of an increase in the size of the abdomen. Three, all with mixed tumors composed largely of striated muscle, had passed portions of the tumor *per vaginam*.

The average duration of symptoms was about 29 weeks in the 29 patients from whom this information was available.

Only 4 patients upon whom follow-up data were available survived more than 2 years. The others died on an average of 33 weeks after the diagnosis was made and 52 weeks after onset of symptoms. This indicates the inadequacy of even the radical hysterectomies that were performed upon almost all of these patients.

Part II

Cases coming under the observation of the writers

Case I

C. C., a white American housewife, 66 years old, was admitted to the New Haven Hospital on December 30, 1936 complaining of vaginal bleeding. This had started 6 months previously and had continued intermittently two to three times a week up to the time of admission. The bleeding was never excessive and had not necessitated the wearing of a pad. During this period the patient felt run down, tired easily and lost 20 pounds in weight.

The menses, which started at the age of 13, came regularly at monthly intervals and lasted 5 days. Menopause occurred at the age of 48 and the patient was free of symptoms until the onset of the present illness in August 1936. The patient was never pregnant although contraceptives were never employed.

The patient was moderately obese and had a blood pressure of 180/100. There was evidence also of cardiac hypertrophy. The lungs were clear to auscultation and percussion. Vaginal examination revealed a soft, smooth cervix with an os which admitted the tip of the finger. The body of the uterus was increased in size but the adnexa were not felt.

On January 4, 1937 the uterus was curetted. The cervix was smooth and devoid of ulcers. The uterus was retroflexed and definitely enlarged. A thickening suggestive of an old inflammatory process was felt in the adnexal regions. The cervix was dilated and the uterine cavity was found to measure 11.4 cm. in depth. Exploration of the cavity with a blunt, serrated curette revealed a zone of softening in the region of the right cornu and a similar less extensive zone on the left. The lining of the remainder of the uterus had a normal consistency. The curettings obtained consisted of vascular, friable tissue definitely suggestive of neoplasm.

The histological diagnosis of the tissue was leiomyosarcoma. Roentgenographic examination of the chest on January 7, 1937 revealed several small, metastatic tumor nodules in both lung fields. On January 18 a total hysterectomy was performed. The uterus was found to be five times normal size and apparently contained a myoma in the fundus. The left ovary was enlarged, cystic and adherent to the pelvic peritoneum. The right ovary was atrophic. The patient was discharged from the hospital on February 3 symptomatically improved. A roentgenogram of the chest 2 days before discharge showed extensive spread of the pulmonary metastases.

At home the patient improved subjectively although she expired 4 months after the onset of disease, apparently from extensive metastatic tumor. Postmortem examination was not obtained.

Gross Notes. The surgical specimen consisted of the entire uterus including the cervix, tubes and ovaries (Fig. 1). The uterus measured 9 cm. in width, 9 cm. in length and 6 cm. anteroposteriorly in the fundic portion, which appeared to be markedly enlarged. The serosal surface was smooth and shining except for a solitary myoma 1 cm. in diameter on the posterior wall immediately above the peritoneal reflection in the cul-de-sac. Both tubes were thin walled and their fimbriated ends were free. The right ovary was a small, firm, yellow, almond-shaped, atrophic organ which measured 3 by 1.5 by 0.8 cm. The left ovary was an enlarged cystic spherical mass which measured 5 by 3.5 by 3.5 cm. Its serosal surface was smooth and shining but somewhat irregular. On cross section this ovary appeared to be completely replaced by soft, yellow, friable tissue forming tiny papillary structures that completely filled the lumen of a cyst. Surrounding the cyst was firm, homogeneous, yellow, fibrous tissue. The left fallopian tube was attached along the surface of this cystic mass. When the uterus was sectioned a large mass was found to occupy

the fundic portion. This replaced most of the uterine musculature in the posterior wall and appeared to arise from almost the entire fundus and posterior wall. It did not appear to extend appreciably into the anterior wall. The mass within the wall measured 4.5 cm. in diameter. It extended into the lumen of the uterus as a polypoid structure which measured 3 cm. in length and 2.5 cm. in diameter. On the lateral walls the polypoid projection was adherent at several points. The apical portion of the mass had a gray brown, opaque, friable surface. In gross appearance the tumor resembled a polypoid submucous myoma. It differed, however, in that it was adherent to the wall at several points and the line of demarcation between the myometrium and the tumor itself was poorly defined. On cross section the tumor presented a surface of interlacing strands of dense white tissue interspersed with a more gelatinous, translucent tissue. The uterine wall in the fundus was thinned to a width of approximately 0.5 cm. in contrast to uninvolved portions which measured approximately 1.5 cm. in thickness. The endometrium below the tumor had a smooth, shining appearance. The cervix was smooth, firm and covered by intact epithelium.

Microscopic Notes. The tumor, microscopically, was found to be composed primarily of cartilaginous tissue in varying degrees of differentiation (Figs. 2–4). It was made up of masses of this tissue separated from one another by strands of spindle-shaped cells which appeared to represent remnants of the myometrium. This cartilage-like tissue had a matrix composed of homogeneous blue staining material, scattered throughout which were cells with basophilic, vesicular nuclei and vacuolated cytoplasm. In many instances these cells were arranged in pairs and the general appearance suggested that of adult cartilaginous tissue. As one approached the periphery of such masses, the blue matrix disappeared and dense cellular tissue was encountered (Fig. 2). The transition from the central to the peripheral portion was gradual. The cells at the periphery were large, and varied from polyhedral to spindle shaped. The nuclei were large, blue staining and vesicular with only a loose reticular framework. The cytoplasm was basophilic. In this peripheral portion numerous cells were found in mitotic division. The matrix in several of the masses stained a deep blue or purple and had a granular appearance indicating

calcification. In some places a pink staining quality of the matrix suggested osteoid tissue although no well formed, bony structure was encountered (Fig. 3). At the periphery of the masses the tumor cells extended in irregular fashion into the surrounding myometrium. In several places at the periphery the tumor lay within endothelium-lined channels which represented either lymphatics or small blood vessels (Fig. 4). The free surface of the tumor which projected into the uterine cavity was necrotic and was covered by an exudate of polymorphonuclear leukocytes. These infiltrated for a short distance into the tumor. There was also extravasation of blood in this portion. The endometrium was completely absent over the distal part of the tumor, and throughout the uterus it was atrophic and composed of occasional simple glands, some of which appeared dilated and lined by a columnar epithelium with basally placed nuclei. The endometrial stroma was almost entirely lacking. The endometrium was reflected onto the surface of the tumor but was at once interrupted by necrosis and ulceration. The myometrium was atrophic, and consisted of small spindle-shaped cells containing small, deeply staining nuclei.

The cyst of the left ovary possessed a dense wall of fibrous tissue about which the usual spindle-shaped, deeply blue staining cells of the ovarian stroma could be identified. The cyst was lined by innumerable papillary projections that branched in complicated fashion to fill the entire lumen. The papillary projections had delicate fibrous stalks covered by a variable, tall columnar epithelium. Many of these cells were in mitotic division. In some portions the epithelial cells appeared to infiltrate the fibrous tissue of the wall irregularly. Extensive necrosis of the epithelium had occurred in several places and here only a granular, amorphous, pink staining debris remained. In other portions fusion of numerous villi gave the tumor an acinar appearance. The fallopian tubes were atrophic structures with simplified villi composed of a central fibrous stalk covered by columnar epithelium.

Discussion. The cartilage resembled closely that of other tumors. Its growth properties *in vitro,* as described in the text, were very much like those of fetal cartilage (Figs. 5–8). This case is also of interest because there was an associated papillary cystadenocarcinoma in the left ovary.

Case II

This patient was a dressmaker of French-Canadian descent and 59 years old at the time of her first admission to the Meriden Hospital. Her chief complaints at this time were of pain in the lower abdomen and of bleeding *per vaginam*. Her menopause had come at 47 years of age, after a normal menstrual life beginning at 13. There was no history of serious illness in the past nor had any operations been performed. Two and one-half months before admission the patient began to have pain in the lower abdomen. The pain was intermittent and was associated with a scanty, sanguineous, vaginal discharge. The attacks gradually became more frequent and the pain more severe until 1 week before entrance into the hospital, when both pain and discharge became continuous.

Physical examination showed nothing of note except a blood pressure of 200/100. Vaginal examination revealed a bleeding, partially necrotic mass extending through the external os. The attachment was in the fundus. The uterus was not enlarged.

On October 11, 1937 the polypoid mass was excised by cutting the pedicle with scissors. When the diagnosis of malignant mixed tumor of the uterus was made, the patient was urged to return for hysterectomy. The uterus, cervix and adnexa were extirpated on November 15. Postoperative recovery was uneventful. She was readmitted on December 19, 1938 complaining of abdominal pain. She had felt well until about 5 weeks before this time, when she was seized with cramplike pains in the abdomen. Her abdomen became distended and she felt nauseated. The chest was clear to percussion and auscultation. In the left flank of the distended abdomen was felt a large mass. Another firm mass was palpated to the left of the vaginal vault. At the exploratory laparotomy that was performed on December 20, numerous exceedingly firm nodules were found in the floor of the pelvis and within the mesentery and omentum. The latter was thick, extended boardlike across the abdomen and was adherent to the liver and adjacent loops of intestine. A nodule was removed from the omentum for histological examination. This contained carcinoma and myxosarcoma but not cartilage. The patient died at home on January 22, 1939. Necropsy was not performed.

First Specimen

(Polypoid mass excised October 11, 1937. M.S.P. No. 4583.)

Gross Notes. The specimen consisted of two masses of tissue. The larger was mushroom shaped and measured 5.5 by 4 by 2.5 cm. The smaller was ovoid with dimensions of 3.5 by 2.5 cm. That part of the larger specimen which corresponded to the head of the mushroom resembled the smaller mass in its gross features. The tissue was soft and was composed of a yellow gray opaque matrix enclosing numerous translucent gray zones which did not exceed 3 mm. in diameter. Hemorrhage had occurred in a small region near the periphery and a rough, irregular dark brown clot was visible on the surface. Minute depressions could be distin-

guished on the cut surface, possibly corresponding to the lumina of glandular structures. The stalk of the mushroom consisted of closely appressed nodules of firm tissue that gave the specimen a bosselated appearance. Within an extremely translucent gray matrix lay whorls of opaque white fibers. This tissue resembled a common fibroid.

Microscopic Notes. Histologically, the protruding mass was remarkable for the diversity of its component elements (Figs. 9–13). There were acini of various types, scattered round and spindle-shaped cells resembling those of sarcoma, and islands of tissue with the characteristics of cartilage. Striated muscle fibers were not found in an extensive search.

The acini, which had no resemblance to the uterine glands, varied in size and shape as did their lining cells. These were cuboidal or columnar cells devoid of cilia. Some of the smallest acini were lined by flattened cells but keratinization was not in evidence. The lining cells as well as those of the interstitium were often found in a state of mitosis and these mitoses frequently were atypical. Only occasionally were eosinophilic nucleoli seen. Chromatin in all cells of the tumor occurred as delicate strands except in a few pyknotic elements. Certain of the interstitial cells had huge polymorphous nuclei within a vaguely defined cytoplasmic mass. The islands of cartilage had, superficially at least, a typical appearance. The matrix was homogeneous and stained deep blue with hematoxylin and green with Masson's Lichtgrün stain. The small nuclei of the cells within this matrix were surrounded by vacuolated, basophilic material. At the margin of the islands spindle-shaped cells grouped themselves concentrically in successive layers. Internally they shaded imperceptibly into the cartilage-like cells. Externally they resembled more and more the interstitial cells. In some places, however, cartilage was not so sharply defined. It appeared to be merely a development of a matrix substance that widely isolated certain elements of the interstitium.

In the junctional zone of preparations stained by the Wilder method the reticulum was found to form a sharp basement membrane for the epithelium but not for the cartilage (Figs. 11 and 12). In the case of the latter, reticulum fibers seemed to fade into the cartilage matrix, often entering perpendicularly to the expected

course of the fibers in a basement membrane. The reticular support of the interstitial elements was very dense and embraced each cell in many places but often small groups of cells were isolated, suggesting that much more of the interstitium may be epithelial than seemed probable at first glance. There was variation in different sections in the proportions of cartilage, acini and interstitium. In some places there was infiltration with small mononuclear cells. Proximally the atypical tissue was not sharply delimited from tissue that had the histological appearance of a myoma. Deeper within the latter there were spaces lined by epithelium resembling very closely the acini seen in the tumor proper. On its external aspect the tumor tissue had become necrotic and was densely infiltrated with polymorphonuclear leukocytes.

Second Specimen

(Excised November 15, 1937. M.S.P. No. 4638.)

Gross Notes. This specimen consisted of a uterus removed intact with cervix and adnexa. It was a small, thin-walled, pear-shaped structure measuring 7 cm. from the serosal aspect of the fundus to the external os, 3.5 cm. transversely and 1.5 cm. anteroposteriorly. All of the serous surfaces were smooth and transparent. The thickness of the myometrium did not exceed 1.5 cm. It consisted in general of a gray, translucent and resilient, fibromuscular tissue embedding thick, firm-walled blood vessels. It was lined for the most part by a shining, exceedingly thin, mucus-covered, gray pink membrane. On the posterior aspect, beginning about 1 cm. from the ostium of the right tube, was an oval, rough, red, elevated zone projecting about 2 cm. into the lumen. A much smaller rough area was found in the wall of the cervical canal posteriorly. Sections were made including these two portions of the uterus. Each ovary was an ovoid structure 2.5 by 1.5 by 1 cm. The capsules were smooth. There was a translucent stroma enclosing many convoluted, more opaque corpora fibrosa. Each tube pursued a slightly convoluted course, was 6 cm. long and terminated in free fimbria.

Microscopic Notes. A section was examined from the wall of the uterus at the elevated, rough, red zone described grossly. This was the apparent site of origin of the tumor. Here a lining mem-

brane consisting of a single layer of tall columnar epithelium was made to project into the lumen by clublike extensions of a rather loose stroma of connective tissue containing dilated capillaries, extravasated red blood cells, and small mononuclear cells. Into the stroma projected irregular glands but the epithelium was only slightly atypical and mitoses were few. The remarkable thing was that such small atypical glands were situated within their myxomatous stroma deep within the myometrium. Often minute buddings of the glands occurred and in other sections from the same region a thick papillary layer of glandular tissue was found. This resembled, in some respects, the epithelium of the cervix; the cytoplasm was abundant and vacuolated and the cells were tall and the nuclei in general had a basal position. The adjacent endometrium was atrophic, the glands were typical but small, and the stroma consisted of dense, spindle-shaped, deeply staining, basophilic elements.

Third Specimen

(Fragment of omentum removed at exploratory laparotomy December 20, 1938. M.S.P. No. 5574.)

Gross Notes. The specimen consisted of a mass of tissue said to be derived from the omentum. Most of it was translucent yellow adipose tissue embedding very firm, gray, translucent masses. The latter offered great resistance to incision.

Microscopic Notes. In the mass removed from the abdomen the tissue was very much like that of the deeply invading glandular tissue of the uterus, but here the acini were more atypical (Fig. 13). These were again embedded in the myxomatous stroma which in turn was surrounded by dense collagen. Mitoses were not common. Although the stroma was myxomatous, actual cartilage was not in evidence.

Discussion. In preparations stained by the Wilder method, epithelium, sarcomatous stroma and cartilage maintained typical relations with the reticulum. That the original tumor and metastases may differ has often been noted.

Case III

The patient was a white woman, 62 years of age, upon whom there was performed an hysterectomy under suspicion of chorionepithelioma. Only

meager clinical data were available. It was stated that for 3 months she had been troubled with a bloody vaginal discharge, severe backache and pain in the lower abdomen. She had her menarche at the age of 16 years and the menopause had occurred suddenly at the age of 55. There had been one miscarriage, but four children were delivered normally.

Bimanual palpation showed the uterus to be firm and about six times the usual size. After removal it was described as soft and boggy. The tumor was stated to have been attached to the fundus.

Gross Notes. Only a small part of the tumor measuring 4 by 5 by 3 cm. was sent to the laboratory for study. About one half of the surface of the specimen was smooth and shining but beneath this surface could be seen extravasated blood. Incision showed the tissue to have a faint pink color and to be traversed by whorls of fibers. All cut surfaces had the same appearance.

Microscopic Notes. Microscopic examination showed the tumor to consist of polymorphous elements (Figs. 14 and 15). These were largely spindle-shaped cells that varied greatly in size. They occurred in interlacing fasciculi. There were large, apparently empty spaces among the cells, probably the result of shrinkage because of poor fixation. Sections stained with Sudan III showed only scanty deposits of fat in the form of fine, intracytoplasmic granules. A few of these cells showed distinct cross striations, particularly in preparations stained by the Wilder silver stain. Such striations were also found in some of the many mononuclear giant cells that were scattered among the spindle-shaped elements. Here the striations formed remarkably complex patterns (Figs. 16–20). The long, vesicular nuclei of the giant cells resembled those of the other cells. Their chromatin was in the form of minute granules which condensed at the periphery to form a distinct but thin nuclear membrane. Single or many brightly acidophilic nucleoli were surrounded by narrow halos of clear nucleoplasm. Mitoses were moderately abundant and often atypical. A few acini lined by cuboidal cells contained a finely granular material that stained with eosin (Fig. 14). Wilder's reticulum stain demonstrated a sharp, delimiting argyrophil membrane upon some aspects of these acini, but elsewhere the lining cells were in contact with, and difficult to distinguish from, the surrounding polymorphous elements. Reticulum fibers occurred also in small wisps intimately enmeshing individual cells elsewhere. There was an abundance of collagenous stroma throughout, as demonstrated in

preparations stained by Masson's Lichtgrün method. Small, poorly demarcated foci of necrosis were seen in a few places but hemorrhages were few. The abundant blood vessels had thin walls and were lined in some places by typical thin endothelial cells, but elsewhere by large cells indistinguishable from those of the tumor. The methods of Bielschowsky and Nissl failed to demonstrate nerve cells in the tumor.

Discussion. This tumor consisted of sarcoma-like tissue, striated muscle cells and epithelium. The last was in some places vaguely delimited from the first, suggesting the possibility of a common derivation in the sense of Masson. Evidence for this was not conclusive. There were no structures undeniably similar to those of the neural crest, and nerve cells could not be demonstrated.

Summary

Mesodermal mixed tumors are among the rarest and most malignant neoplasms of the body of the uterus. They occur almost entirely in women between 45 and 65 years of age and manifest themselves clinically as does carcinoma of the uterus except that abdominal pain is more often a major symptom. Most of these tumors are polypoid and usually take origin either at the cornua or from the posterior wall. Metastases are most often local. No essential difference is noted between tumors containing striated muscle and those consisting in part of cartilage.

The mixed tumors present histological features typical of the various tissues which compose them when studied by means of reticulum and other special stains. In tissue cultures a cartilaginous tumor grows in a pattern characteristic of normal cartilage. These observations, together with the multiplicity of the tissues in many of the tumors, support the theory that pathogenesis depends upon multipotential anlagen rather than upon metaplasia.

Appendix

Cases Previously Reported

Mixed Tumors Composed Chiefly of Cartilage or Bone

Azzola, Fabian. Ein Fall von Sarcoma uteri polymorphocellulare. *Zentralbl. f. Gynäk.*, 1924, **48**, 2285–2287.

Age 47. Prolapse of uterus, pain in back and frequent and painful urination, 6 to 7 weeks. Bosselated tumor, size of child's head, embedded in

posterior wall of uterus. Histology: Islands of cartilage invading veins; polymorphous elements, including giant cells.

Blasek, Stefan (case 1). Knorpeleinschlüsse in der Uterusschleimhaut. *Arch. f. Gynäk.*, 1930, **141**, 539–547.

No clinical data. Histology: Adenocarcinoma, myxomatous stroma, cartilage.

Blasek, Stefan (case 2). *Ibid.*

No clinical data. Histology: Adenocarcinoma, myxomatous stroma, cartilage.

Chavannaz, M. M., and Nadal, Pierre (case 1). Des tumeurs mixtes de l'utérus. *Gynécologie*, 1920, **19**, 3–35.

Age 52. Severe pain in right side of abdomen. Enlarged soft uterus adherent to peritoneum. Springing from left posterior aspect of uterus, apparently from the musculature, was a tumor, size of a fetal head at term. Histology: Sarcoma, angiosarcoma, islands of cartilage, osteoid tissue, adenomyoma, multinucleated giant cells. Death 3½ months after hysterectomy.

Chavannaz, M. M., and Nadal, Pierre (case 2). *Ibid.*

Age 59. Ascites and mass in abdomen, abdominal pain, vaginal bleeding. Unusual nodule arose from posterior wall of uterus. Histology: Cylindrical epithelial cells, sarcoma and cartilage. Three months after radical hysterectomy an inguinal node the size of an egg was removed.

Delagenière, Yves, and Beauchef, P. Tumeur mixte de l'utérus avec métastase tibio-péronière. *Ann. d'anat. path.*, 1927, **4**, 617–620.

Age 54. Hemorrhages from uterus. Lobulated mass in vagina, attached by broad base within body of uterus. Histology: Cartilage, osteoid tissue. Tibia amputated 10 months after total hysterectomy for sarcoma (metastasis?). Death 14 months after hysterectomy.

Durante, G., and Roulland, H. Tumeur embryonnaire maligne de l'utérus (myxo-chondrome. *Gynécologie*, 1924, **23**, 193–211. (Also: *Bull. Soc. d'obst. et de gynéc.*, 1924, **13**, 28–30.)

Age 52. Metrorrhagia lasted from 4 to 6 weeks. Cervix dilated by soft malodorous masses. Abdomen tender and distended. Tumor masses sprang from corpus and cervix. Histology: Myxomatous tissue, including islands of cartilage. Death 4 months after total hysterectomy.

Fels, Erich. Mischtumor des Corpus uteri. *Monatsschr. f. Geburtsh. u. Gynäk.*, 1928, **78**, 279–287.

Age 66. Vaginal discharge with slight bleeding; pains in back for 3 months. Submucous myoma covered by atypical tissue attached to anterior and right lateral wall. Histology: Islands of sarcoma, cartilage, muscle(?), and atypical epithelial cells. Six months after radical hysterectomy and irradiation, recurrence in vaginal pouch. Histology: Carcinoma, no cartilage.

Frankl, Oskar (case 1). Über Koinzidenz und Interferenz von Uterustumoren. I. Myom und Sarcom. *Arch. f. Gynäk.*, 1924, **122**, 554–584.

Age 58. Almost constant vaginal bleeding, 6 months. Necrotic mass projected through external os of cervix. Entire endometrium replaced by necrotic and hemorrhagic polypoid masses. Histology: Round cell sarcoma, islands of cartilage.

Frankl, Oskar (case 2). *Ibid.*
Age 49. Metrorrhagia 4½ years. Uterus size of child's head. Histology: Spindle cells, giant cells, cartilage.

Gaebelein, M. Eine heterologe Mischgeschwulst des Uterus: Myosarcoma myxomatodes et enchondromatodes polyposum uteri. Thesis, Halle, 1909.
Age 50. Metrorrhagia, pain in lower abdomen. Polypoid mass projected through cervical os. Tumor attached to anterior and posterior walls. Histology: Cartilage, myxosarcoma, spindle cells and columnar cell epithelium, not atypical. Recurrence 4 months after radical hysterectomy.

Gebhard, C. Eine Mischgeschwulst des Uterus (Endotheliom mit Fett- und Knorpelgewebe). *Ztschr. f. Geburtsh. u. Gynäk.*, 1903, **48**, 111–121.
Age 56. Watery and sanguineous vaginal discharge, cramplike abdominal pain; feeling of pressure upon bladder. Tumor, size of fist, filled upper part of vagina. Polypoid mass sprang from right tubal angle. Histology: Sarcoma, cartilage, smooth muscle, adipose tissue, glands and endothelium.

Geisler, A. Über Sarkoma uteri. Thesis, Breslau. 1891.
Age 50. Tumor attached to left lateral wall. Histology: Chondromyxosarcoma. Death 2 days postoperative.

Hartfall, Stanley J. (case 1). Chondro-sarcoma of the uterus. *J. Obst. & Gynaec. Brit. Emp.*, 1931, **38**, 593–600.
Age 46. Vaginal discharge, at first serous, then serosanguineous and offensive, 18 months. Vagina filled with necrotic tissue. Polypoid tumor arose in region of right cornu. Histology: Fibrosarcoma with cartilaginous foci. Recurrence after 14 months. Death 23 months after hysterectomy. Metastases to lungs. Histology: Myxomatous tissue and cartilage.

Hartfall, Stanley J. (case 2). *Ibid.*
Age 54. Intermittent pains in lower abdomen ("like labor pains") at intervals of about 3 weeks. Uterus enlarged to that of pregnancy at 7 months. Necrotic tumor expanded uterine cavity. Histology: Partially calcified cartilage and myxomatous foci. Death 6 months after panhysterectomy. Recurrence in pelvis and pleura. Histology: Myxomatous tissue with islands of cartilage.

Hartfall, Stanley J. (case 3). *Ibid.*
Age 66. Irregular vaginal bleeding, at first associated with colicky pain, 12 months. Papillomatous growth on posterior wall of uterine cavity. Histology: Round and spindle cells and small giant cells; islands of cartilage. Patient apparently well for at least 5 years after vaginal hysterectomy.

Hofbauer. Rezidivierender Tumor der Corpusschleimhaut. *Monatsschr. f. Geburtsh. u. Gynäk.*, 1909, **29**, 659–661.

Age 59. Vaginal discharge and occasional aching lower abdominal pain, 1 year. Lobulated polypoid mass inserted near ostium of right tube. Histology: Cartilage, sarcoma, proliferated lymphatic lining cells (probably not malignant), glands.

Jessup, D. S. Mixed tumor of the uterus. *Proc. New York Path. Soc.*, 1913, n.s. **13**, 81–83.

Age 66. No clinical history. Soft, friable tissue protruded from fundus. Spindle and giant cells, islands of cartilage, alveoli and sheets of epithelium. Patient died 10 weeks after panhysterectomy following rupture of intestinal anastomosis.

Kaufmann, E. (case 1). Pathology. (English translation by Reimann, S. P.) P. Blakiston's Son & Company, Philadelphia, 1924.

No metastases at necropsy. Case of diffuse sarcoma of endometrium with cartilaginous inclusions.

Kaufmann, E. (case 2). *Ibid.*

Age 72. No clinical history. Tumor size of man's head. Histology: Fibromyoma, myxosarcoma, cartilage, bone and glands.

Kistler, Gene H. A papillary mixed tumor of the body of the uterus. *Am. J. Cancer*, 1932, **16**, 399–411.

Age 64. Vaginal bleeding 3 weeks; pain in lower abdomen 1 day. Mass of opaque gray tissue projected into uterine cavity from attachment upon left and fundic walls. Histology: Adenocarcinoma, islands of cartilage. No evidence of recurrence 9 months after removal of uterus and adnexa and deep X-ray irradiation.

Kleine, H. O. Erstmalige Beobachtung eines Neuroms in der Uteruswand (kombiniert mit einer heterologen mesodermalen Mischgeschwulst). *Arch. f. Gynäk.*, 1931, **147**, 680–687.

Age 44. Menorrhagia, several months. Large gray mass, occupying most of uterus, seemed to arise from myometrium of fundus. Histology: Spindle cells, myxomatous elements, cartilage. Smaller egg-sized yellow mass which did not seem to infiltrate the former. Histology: Neuroma composed of myelinated nerve fibers. No evidence of recurrence 2 years after removal of uterus and adnexa.

Köhler, R. Myxochondrosarcoma uteri. *Zentralbl. f. Gynäk.*, 1919, **43**, 113–115.

Age 67. Vaginal bleeding, 2 months. Ovoid necrotic tumor mass in vagina. Numerous pillow-like masses projected from wall of uterus everywhere. Histology: Undifferentiated cells, myxomatous tissue, cartilage.

Lahm, W. Heterologe Tumorbildungen des Müllerschen Ganges im Bereich der Cervix und des Corpus uteri (Mischtumoren). In: Halban, J. and Seitz, L. Biologie und Pathologie des Weibes, IV. Urban and Schwarzenberg, Berlin, 1928, p. 652.

No clinical data. Polypoid mass attached in region of left tubal ostium. Histology: Carcinoma, myxochondrosarcoma.

Malapert, P., and Morichau-Beauchant, R. Tumeur conjonctive mixte (myxo-chondro-sarcome) de l'utérus. *Bull. et mém. Soc. anat. de Paris*, 1905, **80**, 391.

Age 41. Uterine hemorrhages; colicky pain in abdomen. Uterus enlarged and vagina filled by friable mass; exact attachments not known. Histology: Spindle cell sarcoma; many nodules of cartilage. Death 13 months after onset of bleeding following total removal of the intravaginal mass. Local recurrence of this; uterus greatly enlarged.

McDonald, John R., Broders, Albert C., and Counseller, Virgil S. Sarcoma of the endometrial stroma. *Surg., Gynec. & Obst.*, 1940, **70**, 223–229.

Age 61. No clinical data. Histology: Fibrochondrosarcoma. Lived at least 1 year.

Murray, H. Leith, and Littler, R. Meredith. A case of "mixed tumour" of the uterus (adeno-chondro-sarcoma). *J. Obst. & Gynaec. Brit. Emp.*, 1914, **25**, 26–30.

Age 46. Malodorous, watery, vaginal discharge. Soft polypoid growths replacing endometrium. Histology: Glands, sarcomatous tissue, islands of cartilage.

Nicholson, G. W. Studies on tumour formation. VIII. The mixed tumours. *Guy's Hosp. Rep.*, 1924, **74**, 81–108.

No clinical data. Histology: Cartilage and columnar cells.

Olinder, Ragnar. A case of malignant mixed tumour in the uterus. *Acta path. et microbiol. Scandinav.*, 1933, suppl. **16**, 314–321.

Age 52. Sanguineous vaginal discharge, 5 months. Uterus hard, uneven and about size of 2 months' pregnancy. Tumor in posterior wall seemed to replace myometrium in part. Histology: Cartilage, myxomatous and "cytogenic" tissue. Local recurrence 7 months after hysterectomy. No roentgenographic evidence of pulmonary metastases at this time.

Penkert, M. Eine teratoide Mischgeschwulst des Uterus. (Carcinoma corporis uteri polyposum mit myxomatösem, sarkomatösem, und knorpeligem Stroma). *Beitr. z. Geburtsh. u. Gynäk.*, 1905, **9**, 488–499.

Age 62. Sanguineous vaginal discharge; occasional pains in lower body. Large, tense mass projected above pubis. Uterus 12 by 8 by 6 cm. Polypoid tumor attached to anterior wall. Histology: Islands of cartilage, epithelial nests and cysts, giant cells, endothelium(?). No evidence of metastases at time of radical hysterectomy.

Perlstein, Isidor. The mesodermal mixed tumors of the uterus. Report of a case of botrioid chondrosarcoma of the endometrium. *Surg., Gynec., & Obst.*, 1919, **28**, 43–55.

Age 54. Pain in lower abdomen and back, vaginal discharge for 1½ years. Botryoid yellow masses replaced endometrium except on anterior wall. Histology: Cartilage and stroma of round cells. Ultimately recurred.

Petersen, A. J. (case 1). Mixed tumors of the uterus. *J. Lab. & Clin. Med.*, 1922–23, **8**, 369–374.

Age 60. Tumor 8 cm. in diameter; many mucosal polyps. Histology:

About 2 per cent bone, 35 per cent hyaline cartilage, 35 per cent smooth muscle, 30 per cent connective tissue, 1 per cent alveoli of round and spindle-shaped cells.

Petersen, A. J. (case 2). *Ibid.*
Age 54. Histology: One per cent cartilage and smooth muscle, 5 per cent fibrous connective tissue, 94 per cent fatty areolar tissue. Patient well 2 years after operation.

Rankin, Fred W., and Broders, Albert C. (case 1). Primary fibromyxochondrosarcoma of endometrial stroma. *Am. J. Surg.*, 1931, **12**, 74–75.
Negress, aged 36. Menorrhagia and leukorrhea for 18 months. Uterus three times normal size. Originating in the posterior endometrial wall upon a broad base, was a gelatinous and cartilaginous cauliflower-like growth that slightly infiltrated the myometrium. Histology: Oval and stellate cells; fibrous and myxomatous tissue, cartilage.

Reid, W. L. Notes on a case of chondrosarcoma of the uterus. *Glasgow M. J.*, 1902, n.s. **57**, 371–374.
Age 59. Copious, malodorous vaginal discharge, 4 months; sanguineous discharge, 2 months. Uterus enlarged to size of 4 months' pregnancy. Cavity filled by lobulated tumor. Histology: Myxomatous tissue; islands of cartilage.

Reinecke, Hans. Drei verschiedenartige heterologe mesodermale Kombinationsgeschwülste des Uterus. *Ztschr. f. Geburtsh. u. Gynäk.*, 1933, **104**, 140–157.
Age 66. Uterine hemorrhage, 6 months. Uterus 13 by 7 by 8.5 cm. Attached by broad base in right tubal angle upon a myoma was a soft polypoid mass. Histology: Cartilage, undifferentiated sarcomatous stroma, adenocarcinoma, giant cells. Recurrence in retroperitoneum 9 months, and death 11 months, after total hysterectomy and X-ray therapy.

Ritter, Otto. Über einen mesenchymalen Mischtumor des Uteruskörpers. *Ztschr. f. Geburtsh. u. Gynäk.*, 1926, **89**, 266–271.
Age 58. Uterine bleeding for several weeks. Histology of curettings: Edematous fibrillar tissue, polymorphous cell sarcoma, osteoid tissue. Uterus, removed later, 8 by 5.5 by 4 cm. Histology: Epithelium, small-celled masses resembling blastema of Wilms's tumor.

Schröder, R., and Hillejahn, A. Über einen heterologen Kombinationstumor des Uterus. *Zentralbl. f. Gynäk.*, 1920, **44**, 1050–1058.
Age 58. Severe metrorrhagia, 3 weeks. Polypoid mass attached 2 cm. above internal os. Histology: Cartilage, carcinoma, fatty tissue, "perithelioma," nervous tissue. Ovary the seat of a papillary tumor. Death 13 months following radical hysterectomy and irradiation.

Seydel, Otto. Ein Enchondrom des Uterus. Ein Beitrag zur Genese der Mischtumoren des Uterus. *Ztschr. f. Geburtsh. u. Gynäk.*, 1901, **45**, 237–271.
Metrorrhagia, 3 months. Tumor mass filled vagina and was attached by broad base between corpus and cervix. Cartilage with sarcomatous(?) spindle cell stroma.

Sophian, Lawrence (case 1). Adenosarcoma of body of uterus. *Am. J. Obst. & Gynec.*, 1932, **24**, 911–914.
Age 55. Backache and urinary urgency. Uterus much enlarged. Dilated cavity filled by pedunculated mass. Histology: Carcinoma, myxomatous stroma, cartilage, smooth muscle. Death 14 months after removal of uterus and adnexa.

Sophian, Lawrence (case 2). *Ibid.*
Age 64. Vaginal bleeding, 4 months. Lumen filled by yellow fungating, pedunculated growth attached to fundus. Histology: Carcinoma, myxomatous stroma, cartilage, smooth muscle. No evidence of recurrence 11 months after hysterectomy and removal of adnexa.

Stout, A. P. Human Cancer. Lea & Febiger, Philadelphia, 1932.
Age 50. Menorrhagia for 30 months ending with foul discharge. Uterus the size of pregnancy at term. Histology: Huge masses of hyaline cartilage, undifferentiated cells, papillary epithelium.

van Akkeren, R. Zwei seltene Fälle von Gebärmuttergeschwulst. *Zentralbl. f. Gynäk.*, 1930, **54**, 905–913.
Age 60. Scanty, odorless, vaginal discharge 5 weeks; urgency and dysuria, 4 weeks. Suprapubic mass extended to 3 cm. below the umbilicus. Friable mass filled the entire uterine cavity. Histology: Cartilage. Recurrence 3 months after hysterectomy, X-ray irradiation. Necropsy 10 months postoperative. Tumor in bones, liver and lungs. Histology: Sarcoma with giant cells, no cartilage.

Wagner, E. Verjauchende Enchondrome des Uterus, Lungenenchondrome, frische Peritonitis. Der Gebärmutterkrebs. Leipzig, 1854, p. 129. (Cited in Williams, J. Whitridge. *Am. J. Obst.*, 1894, **29**, 721–764.)
Age 55. Thin-walled intra-uterine cyst from whose inner surface many cartilaginous villi projected. About fifteen nodules with similar structure in each lung. Histology: Hyaline cartilage, spindle-shaped and star-shaped elements.

Wiener, Solomon. A mixed cell tumor of the uterus. *Am. J. Obst. & Gynec.*, 1924, **8**, 211–215.
Age 50. Vaginal bleeding; cramplike abdominal pain. Polypoid mass protruded into vagina through the cervical canal. Histology: Fibromyxochondroma contained islands of glandular tissue, some not of endometrial type. Hysterectomy 3 months after polypectomy. Huge polypoid mass projected into uterine cavity from all sides.

Wolfe, Samuel A. Mixed tumor of the body of the uterus. *Am. J. Obst. & Gynec.*, 1930, **19**, 816–822.
Age 55. Sanguineous vaginal discharge; tender, irregular mass in abdomen extended to umbilicus. Large uterine cavity filled with tuberous mass arising from posterior, anterior and lateral walls. Histology: Cartilage, osteoid tissue, smooth muscle and spindle cells.

Mixed Tumors Containing Striated Muscle

Amolsch, A. L. Mixed mesodermal tumors of the uterus and vagina. With report of six cases. *Am. J. Cancer*, 1939, **37**, 435–444.

Age 57. Vaginal bleeding, pain in lower abdomen and slight enlargement of lower abdomen, 2 years following removal of "glandular endometrial polyp" attached high in uterus. At laparotomy, uterus greatly enlarged and miliary metastases upon peritoneum. Large polypoid mass sprang from posterolateral endometrium at cornu of uterus. Histology: "Polymorphous malignant stroma," cartilage, bone, striated muscle.

Anderson, A., and Edmansson, Ernst. Rhabdomyoma und mehrere andere Geschwülste in einem Uterus. Nord. medic. Arkiv., Bd. 1, No. 4. (Quoted in: *Jahresb. ü. d. Leistung. in d. ges. Med.*, 1869, **4**, 187–188.)
Age 50. Vaginal discharge for several years. Vagina filled with large soft tumor that had finger-shaped extensions. Attachment at junction of cervix and corpus(?) Histology: Striated muscle, cysts lined with flattened epithelium. Death 2 months after removal of uterus.

Bystroumow, and Eckert. Rudnew's Journal, 1874, p. 442. (Cited in Kolessnikow, N. Pigmentirtes Rhabdomyom (Rhabdomyoma melanodes). *Virchows Arch. f. path. Anat.*, 1876, **68**, 554–575.)
No clinical data. Pedunculated tumor attached to endometrium. Histology: Striated muscle, binucleate or trinucleate spindle-shaped cells.

Colomiatti, V. Contribuzione allo studio dei tumori dell' utero. *Arch. per le sc. med.*, 1881, **5**, 1–23. (Quoted by Robertson.)
No clinical data. Tumor, size of fetal head, probably arose from the corpus. Histology: Large round cells, striated muscle.

Frank, R. T. Gynecological & Obstetrical Pathology. D. Appleton & Company, New York, 1922.
Age 70. No clinical history. Histology: Carcinoma, squamous epithelium, embryonal striated muscle.

Frankl, Oskar (case 3). Über Koinzidenz und Interferenz von Uterustumoren. I. Myom und Sarkom. *Arch. f. Gynäk.*, 1924, **122**, 554–584.
Age 47. Menorrhagia and dysuria. Necrotic and hemorrhagic cauliflower-like mass sprang from posterior wall of uterus. Histology: Striated muscle, cartilage, osteoid tissue, "atypical stroma."

Gamper, Alfred. Beitrag zur Kenntniss der mesodermalen Mischgeschwülste des Uterus. *Arch. f. Gynäk.*, 1926–27, **129**, 878–890.
Age 54. Yellow vaginal discharge for 16 weeks, blood stained for 4 weeks. Soft friable mass protruded from external os of cervix. Uterus 10 by 8 by 7 cm. Endometrium was the source of masses arising everywhere but from anterior wall. Histology: Rhabdomyosarcoma, cartilage, occasional glands, spindle cell stroma. Patient in good health 4 years after hysterectomy.

Glynn, Ernest, and Bell, W. Blair (case 1). Rhabdomyosarcoma of the uterus. *J. Obst. & Gynaec. Brit. Emp.*, 1914, **25**, 1–12.
Age 62. Bloody vaginal discharge for 4 months. Passage of mass the size of an orange *per vaginam*. Ovary the seat of a columnar cell carcinoma. Uterus the size of a 3 months' pregnancy. Polypoid tumor arose from posterior wall of uterus. Histology: Rhabdomyosarcoma with stroma of oval or spindle-shaped cells. Six months later pulmonary symptoms suggested metastases.

Glynn, Ernest, and Bell, W. Blair (case 2). *Ibid.*

Age 75(?). Scanty blood-tinged vaginal discharge (3 months); passed large mass *per vaginam*. Polypoid growths attached to posterior wall. Histology: Striated muscle cells, small round and spindle-shaped cells, multinucleated cells. Recurrence in abdomen 2 months after radical hysterectomy. Histology: Myxosarcoma.

Gunning, R. E. Lee, and Ross, Charles A. Rhabdomyosarcoma of the corpus uteri. *Surg., Gynec. & Obst.*, 1940, **70**, 230–233.

Age 58. Passage of blood clots and foul-smelling discharge *per vaginam*. External os full of necrotic tissue and blood. Nodular gray gelatinous mass originated from the posterior and lateral walls. Histology: Almost all cells of the tumor show cross striations.

Halter, Gustav. Heterotoper Mischtumor des Corpus uteri. *Zentralbl. f. Gynäk.*, 1926, **50**, 2194–2196.

Age 41. Menorrhagia for 12 years. Ten months before examination had a severe hemorrhage followed by passage of "myoma" *per vaginam*. Vagina filled with necrotic, polypoid masses. Uterus size of 5 months' pregnancy. Polypoid mass attached by broad base to posterior wall of corpus. Histology: Striated muscle, glands lined by simple cuboidal epithelium (did not look like carcinoma), embryonal connective tissue. Patient well 3 months following total hysterectomy.

Herb, Isabella C. Mixed tumors of the uterine body. *Surg., Gynec. & Obst.*, 1910, **10**, 463–467. (Also, more briefly, in *Tr. Chicago Path. Soc.*, 1909-12, **8**, 5-7.)

Age 55. Pelvic discomfort for 8 months; vaginal discharge, at times hemorrhagic. Uterus 10 by 7.5 by 6.5 cm. Firm mass, filling entire uterine cavity and penetrating the wall, arose from fundus and region of the right fallopian tube. Histology: Smooth and striated muscle, multinucleated cells, cylindrical cells and round and polygonal cells. Local recurrence with death 6 months after supracervical hysterectomy and right salpingo-oophorectomy.

Hunziker, Hans. Die Rhabdomyome des Corpus uteri. *Beitr. z. Geburtsh. u. Gynäk.*, 1908, **12**, 317–337.

Age 58. Painless vaginal discharge, 5 to 6 weeks. Pelvic mass extended 1 to 2 cm. above symphysis pubis. Mass 6 by 4 by 5 cm., attached to anterior endometrium near ostium of left tube, dilated the uterine cavity. Histology: Round and spindle cell stroma, striated muscle, epithelium that did not have a malignant appearance. Death 5 months after hysterectomy with recurrence in pelvis. Histology: Striated muscle, no cartilage.

Läwen, A. Über ein Rhabdomyosarkom des Uterus mit drüsigen Wucherungen. *Beitr. z. path. Anat. u. z. allg. Path.*, 1905, **38**, 177–206.

Age 60. Slight vaginal bleeding. Uterus size of child's head. Polypoid masses, arising from fundus, posterior wall and ostium of right fallopian tube, involved also the cervix. Histology: Adenocarcinoma, spindle cell sarcoma, striated muscle. Death 1½ years after panhysterectomy, with evidence of peritoneal metastases and peritonitis.

Lochrane, C. D. Rhabdomyosarcoma of corpus uteri. *Proc. Roy. Soc. Med.*, 1933, **26**, 1429–1435.

Age 56. Exsanguination from vaginal hemorrhages of few weeks' duration. Firm friable mass protruded through dilated cervical canal. Uterus size of 10 months' pregnancy. Mass attached to anterior wall of uterus just above internal os. This was locally removed; then hysterosalpingo-oöphorectomy. Histology: Rhabdomyosarcoma.

Reeb, and Oberling, Ch. Rhabdomyosarcome et épithélioma cylindrique du corps utérin. (Dysembryome de l'ovaire droit). *Gynéc. et obst.*, 1929, **19**, 81–90.

Age 51. Profuse uterine bleeding and foul discharge for 1 month. Uterus size of 8 months' pregnancy. Soft masses filled vagina. Polypoid mass sprang from fundus. Histology: Giant cells, striated muscle cells, adenocarcinoma, undifferentiated sarcoma. Metastasis in peritoneum. Histology: Rhabdomyosarcoma. Dermoid cyst in right ovary. Total hysterectomy. Vaginal recurrences 2 months postoperative and death several weeks later.

Robertson, A. Rocke. Rhabdomyosarcoma of the uterus; with the report of a case. *J. M. Research*, 1909, **20**, 297–309.

Age 69. Slight bloody vaginal discharge for 6 months. Red, hard, immovable mass filled upper part of vagina. Source of this was lower portion of corpus and cervix. Histology: Large multinucleated cells; striated muscle cells. No evidence of metastases at necropsy 2 months later.

Shapiro, Phillip F. Rhabdomyosarcoma of the corpus uteri. *Am. J. Obst. & Gynec.*, 1931, **21**, 83–91.

Age 52. Dull pains in lower abdomen for 2 months; nausea, weakness, loss of weight and fever for 2 weeks. Death 24 hours after admission. Uterus enlarged to 18 by 14 by 8.5 cm. by submucosal myoma and by a boggier subserous mass between this and the left ovary. Histology: Rhabdomyosarcoma. No metastases at necropsy.

von Franqué, Otto. Ueber Sarcoma uteri. *Ztschr. f. Geburtsh. u. Gynäk.*, 1899, **40**, 183–243.

Age 49. Irregular menorrhagia for 4 years. Increase in size of abdomen. Abdominal pain and foul-smelling vaginal discharge, 2 weeks. Abdominal mass the size of man's head; soft tumor masses palpable through patulous cervical canal. Soft mass arose from posterior and left walls of uterus. Histology: Round and spindle-shaped cells; smooth and striated muscle fibers. No evidence of metastases at necropsy 1 week after total abdominal hysterectomy.

Additional References

Andervont, H. B. Pulmonary tumors in mice. VII. Further studies on the serial transmission of lung tumors occurring in inbred mice. *Pub. Health Rep.*, 1939, **54**, 1519–1524.

Ascher, S. Zur Casuistik der Myomoperationen. *Ztschr. f. Geburtsh. u. Gynäk.*, 1890, **20**, 307–338.

Bunten, J. C. Sarcoma of the uterus. Report of a case with review of the literature. *Surg., Gynec. & Obst.*, 1925, **41**, 477–480.

Claessen, M., and Mathias, E. Beiträge zur Lehre vom Carcinosarkom. *Beitr. z. klin. Chir.*, 1921, **123**, 584–599.

Feuchtwanger, J. Ein Uterusmyom mit Knorpel- und Knochenbildung. Inaugural dissertation, Strassburg, 1897.

Frankl, Oskar. The coincidence and interference of uterine tumors. *Am. J. Obst. & Gynec.*, 1925, **9**, 745–757.

Girode. Fibres musculaires striées dans une paroi utérine. *Semaine Méd.*, 1892, **12**, 48.

Hellendall, Hugo. Ein intramurales Teratom des Corpus uteri mit Durchbruch in die Uterushöhle und Haarabgang durch die Scheide. *Zentralbl. f. Gynäk.*, 1930, **54**, 2398–2408.

Kworostansky, P. Chondrofibrom des Uterus. *Beitr. z. path. Anat. u. z. allg. path.*, 1902, **32**, 117–145.

Lackner, Julius E., and Krohn, Leon. Report of a case of teratoma of the uterus. *Am. J. Obst. & Gynec.*, 1933, **25**, 735–741.

Manheims, Perry J. Carcino-sarcoma of the uterus. *Proc. New York Path. Soc.*, 1923, n.s. **23**, 74–78.

Mann, W. Zwei seltene Geschwülste des Corpus uteri mit Bemerkungen zu ihrer Entstehungsweise (dreiblättriges, solides Teratom und medulläres Osteochondrom). *Virchows Arch. f. path. Anat.*, 1929, **273**, 663–692.

Masson, P. The rôle of the neural crests in the embryonal adenosarcomas of the kidney. *Am. J. Cancer*, 1938, **33**, 1–32.

Mathias, E. See discussion of von Küttner.

Meikle, G. Jamieson. Mesodermal mixed tumours of the uterus. *J. Obst. & Gynaec. Brit. Emp.*, 1936, **43**, 821–864.

Meyer, Robert. Über Befunde von Knorpel und Knochen im Bereiche der weiblichen Geschlechtsorgane, insbesondere über intraperitonealen Knorpel in Verwachsungsmembranen an den Adnexen. *Virchows Arch. f. path. Anat.*, 1930, **275**, 738–764.

Meyer, R. Über embryonale Gewebseinschlüsse in den weiblichen Genitalien und ihre Bedeutung für die Pathologie dieser Organe. *Ergebn. d. allg. Path. u. path. Anat.*, 1903, **9**, 518–705.

Mönckeberg, J. G. Über heterotope mesodermale Geschwülste am unteren Ende des Urogenitalapparates. *Virchows Arch. f. path. Anat.*, 1907, **187**, 471–516.

Nehrkorn, Alex. Quergestreifte Muskelfasern in der Uteruswand. *Virchows Arch. f. path. Anat.*, 1898, **151**, 52–62.

Pierson, Hannah. Weitere Follikulinversuche. Perforierende Plattenepi-

thelwucherungen im Uterus des Kaninchens mit Knorpel- und Knochenbefunden. *Ztschr. f. Krebsforsch.*, 1938, **47**, 1–12.

Pietzold, G. Zur Kasuistik des Vorkommens von Knorpelgewebe in Uterustumoren. Inaugural dissertation, Leipzig, 1910.

Piquand, G. Le sarcome de l'utérus. Étiologie. — Anatomie pathologique du sarcome du corps. *Rev. de gynéc. et de chir. abd.*, 1905, **9**, 387–446.

Saltykow, S. Beiträge zur Kenntnis des Karzinosarkoms. *Verhandl. d. deutsch. path. Gesellsch.*, 1914, **17**, 351–363.

Seydel, Otto. Ein Enchondrom des Uterus. Ein Beitrag zur Genese der Mischtumoren des Uterus. *Ztschr. f. Geburtsh. u. Gynäk.*, 1901, **45**, 237–271.

Shaw, Wilfred. Mixed tumours of the uterus and vagina. *J. Obst. & Gynaec. Brit. Emp.*, 1928, **35**, 498–513.

Stone, L. S. Experiments showing the role of migrating neural crest (mesectoderm) in the formation of head skeleton and loose connective tissue in *Rana palustris*. *Arch. f. Entwcklngsmechn. d. Organ.*, 1929, **118**, 40–77.

von Küttner, O. Zur Frage der Umwandlung von Uterusmyomen in Sarkom. *Monatsschr. f. Geburtsh. u. Gynäk.*, 1925, **71**, 177–180.

Wilms, M. Die Mischgeschwülste. I: Die Mischgeschwülste der Niere. A. Georgi, Leipzig, 1899. II: Die Mischgeschwülste der Vagina und der Cervix uteri. A. Georgi, Leipzig, 1900.

DESCRIPTION OF PLATES

Plate 1

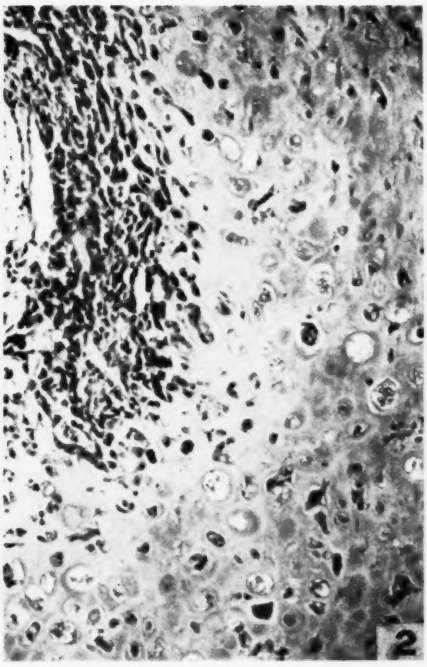
Fig. 1. Case I. Gross specimen in sagittal section. Almost actual size.

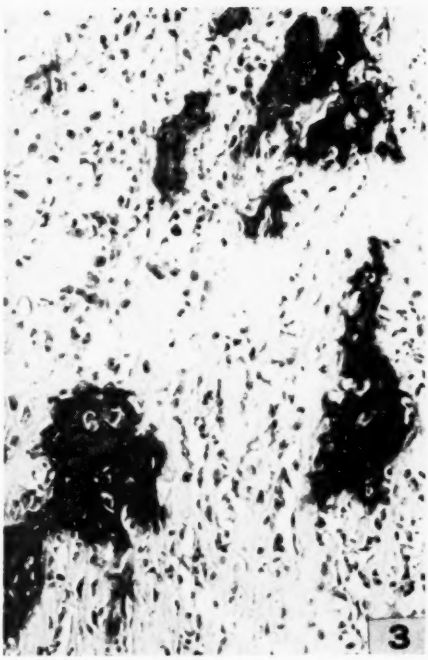
Fig. 2. Case I. Cartilage and spindle-shaped cells. × 125.

Fig. 3. Case I. Cartilage and osteoid tissue. × 125.

Fig. 4. Case I. Tumor in vascular space. × 125.


1


2


3


4

PLATE 2

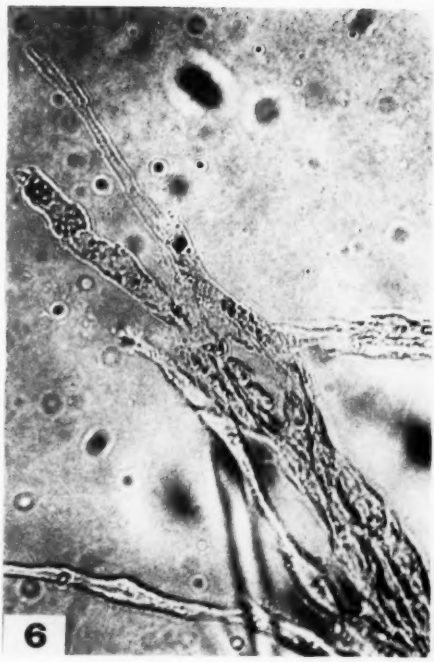
FIG. 5. Human fetal cartilage from head of femur. × 95.

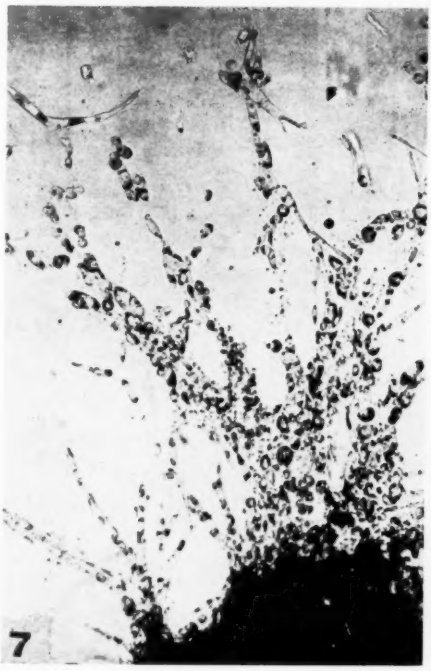
FIG. 6. Higher magnification of a portion of the same culture as shown in Figure 5. × 300.

FIG. 7. Case I. Explant from cartilaginous mass. × 95.

FIG. 8. Case I. Higher magnification of a portion of the same culture as shown in Figure 7. × 300.


5


6


7

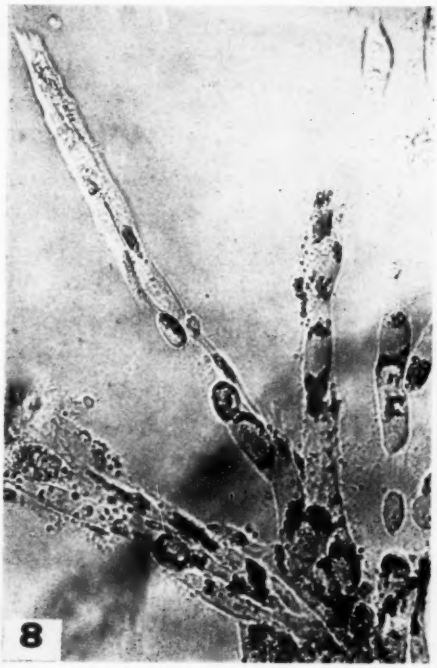
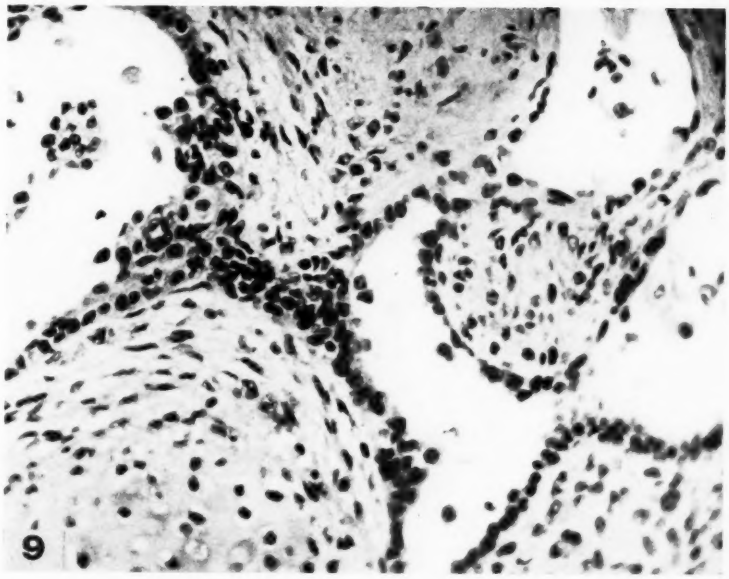
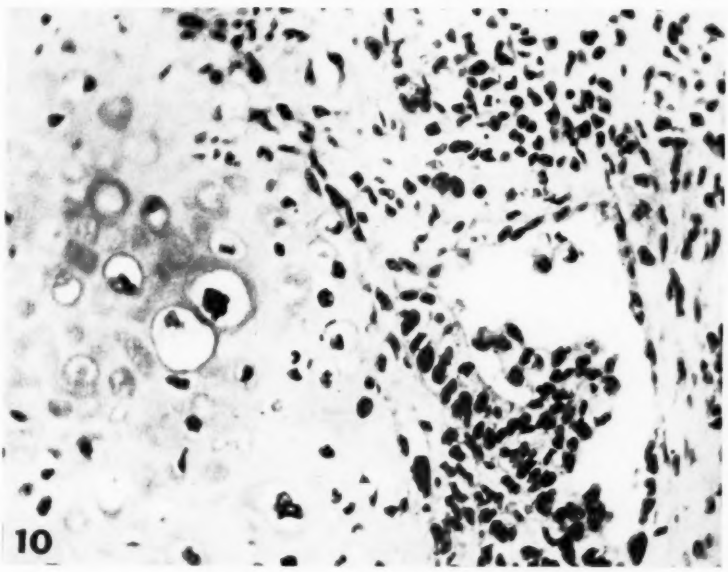

8

Plate 3

Fig. 9. Case II. Mixed tissues at junctional zone. × 235.

Fig. 10. Case II. Epithelium, sarcomatous stroma and cartilage. × 235.





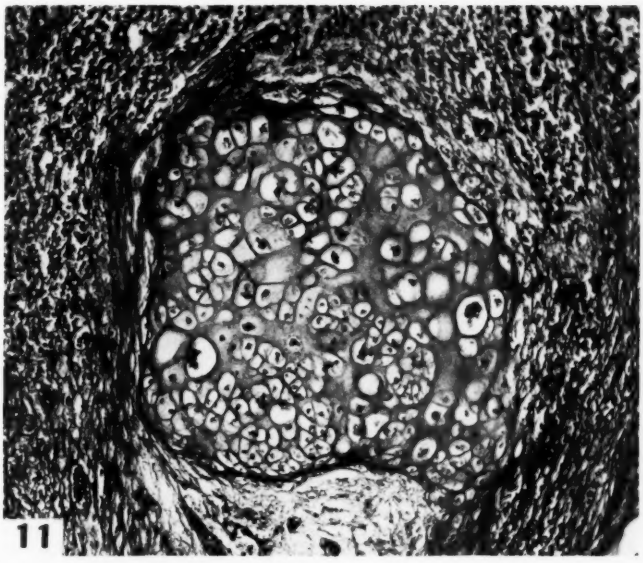
Liebow and Tennant Mesodermal Mixed Tumors of the Uterus

PLATE 4

FIG. 11. Case II. Relation of reticulum to cartilage as shown in a preparation stained by the Wilder method. × 100.

FIG. 12. Case II. Basement membranes of acini as shown in a preparation stained by the Wilder method. × 235.

FIG. 13. Case II. Metastasis in omentum; adenocarcinoma in myxomatous stroma. × 235.


11


12

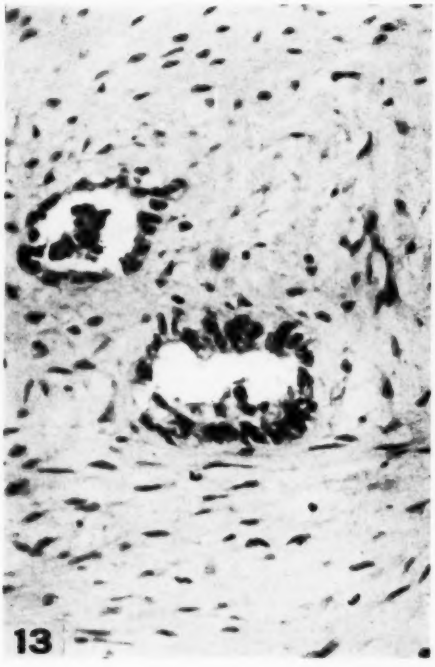
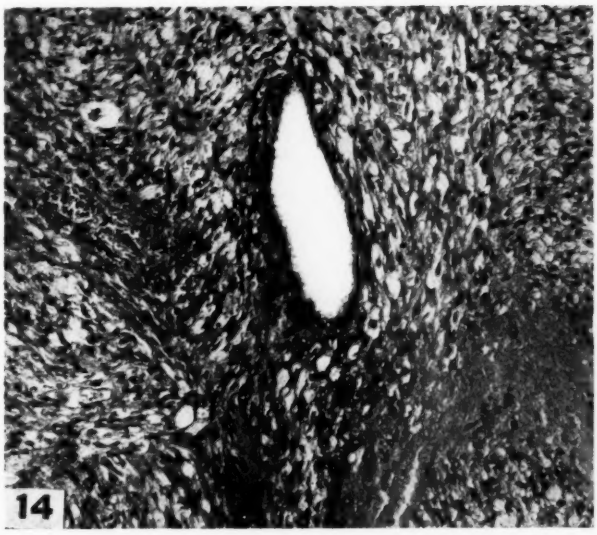
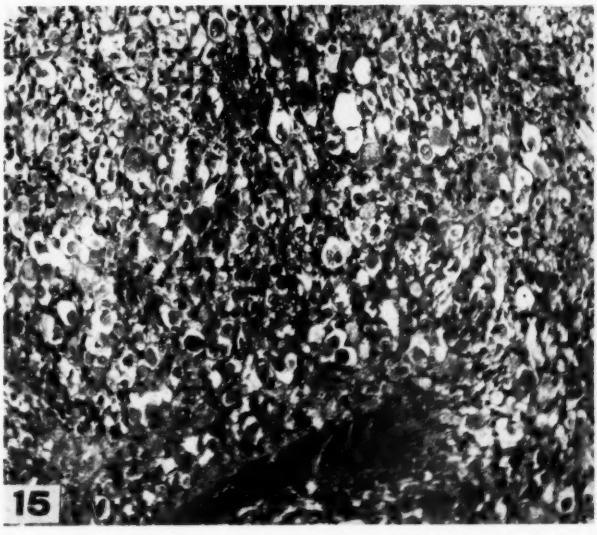

13

PLATE 5

FIG. 14. Case III. Acinus and spindle-shaped cells. Foci of necrosis and hemorrhage. × 125.

FIG. 15. Case III. Giant cells. × 125.

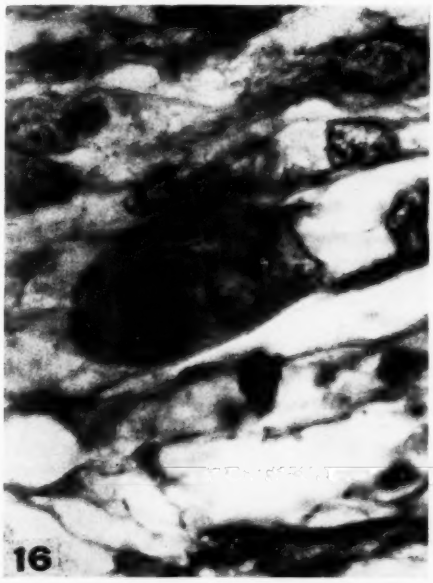


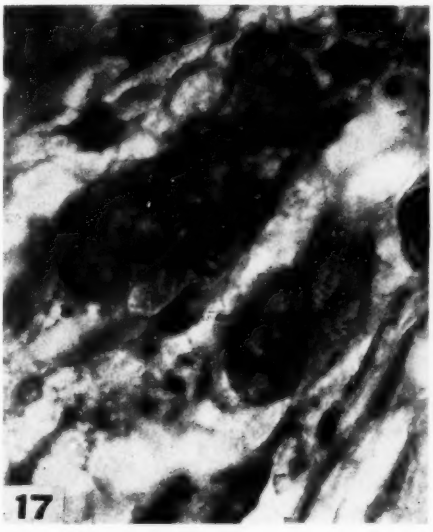


Liebow and Tennant Mesodermal Mixed Tumors of the Uterus

PLATE 6

FIGS. 16. 17. 18. 19. 20. Case III. Striated muscle fibers. × 1000.





Liebow and Tennant

Mesodermal Mixed Tumors of the Uterus

EXPERIMENTAL HYPERTENSION AND PREGNANCY IN DOGS*

JAMES R. DAWSON, JR., M.D., RALPH D. CRESSMAN, M.D., AND ALFRED BLALOCK, M.D.

(From the Departments of Pathology and Surgery, Vanderbilt Medical School, Nashville, Tenn.)

Goldblatt, Lynch, Hanzal and Summerville [1] found that constriction of the renal arteries may result in a sustained elevation of the blood pressure. Dill and Erickson [2] stated that the production of renal ischemia in pregnant dogs or rabbits resulted in an eclampsia-like syndrome, characterized by a rapidly fatal course and significant pathological lesions in the kidneys and liver. In earlier unreported experiments, Mason, Harrison and Blalock noted that pregnant dogs with a preëxisting hypertension due to renal ischemia had a temporary decline in blood pressure without evidence of uremia or eclampsia at the terminal part of the pregnancy period. Similar observations have been made by Goldblatt on dogs and by Williams and Harrison on rats.

METHODS AND RESULTS

The method of Goldblatt and co-workers [1] for producing hypertension was employed, the blood pressure being determined by the direct needle puncture method.

Thirty-one animals were used in this study. Constriction of the renal arteries was produced in 26, 1 animal had a spontaneous hypertension, 2 animals aborted without renal artery constriction having been produced, and hepatic artery occlusion was carried out on the 2 remaining animals. Of the 26 animals with the constriction of renal arteries, abortion or resorption of fetuses occurred in 15, 9 gave birth to live puppies, and 2 died from uremia without either abortion or delivery having occurred.

Of the 15 animals in which abortion or resorption of fetuses occurred, 8 were killed about 24 hours after vaginal discharge was

* Aided by a grant from the Division of Medical Sciences of the Rockefeller Foundation.

Received for publication May 25, 1940.

noted in order to obtain tissues for examination. All of these had presented at least a moderate elevation of blood pressure which in most instances declined slightly at the time of abortion. None of them had convulsive-like movements and all appeared to be in good condition. Of the remaining 7 animals in this group, 4 died after intervals varying from 4 to 13 days following constriction of renal arteries and from 3 to 14 days following abortion. The duration of the pregnancy at the time the clamps were applied was 3, 6, 7 and 7 weeks. A definite elevation in pressure followed constriction of the renal arteries. Death was due to uremia, all having a definite elevation of nonprotein nitrogen. The remaining 3 animals survived following abortion which occurred in 3 days, 3 weeks and 3 weeks following the constriction of the arteries. The duration of pregnancy at the time of application of the clamps was 1, 2 and 4 weeks respectively. All showed a moderate elevation in blood pressure which declined slightly at the time of abortion and rose slightly subsequently. These animals did not appear ill at any time.

Postmortem examinations were performed on the 8 dogs which were killed following abortion and on the 4 which died after abortion. These examinations revealed changes which corresponded to the acute lesions which have been described in nonpregnant animals following constriction of renal arteries; the necrotizing arteriolitis was widespread and it was associated with multiple infarcts of various organs in many of the dogs. In addition to these lesions which are commonly found in acute experimental hypertension, hepatic lesions were present in 4 dogs (3 of 8 which were killed and 1 of those which died). These lesions were not visible grossly and except for slight fatty metamorphosis and some congestion the livers of all dogs appeared normal. Microscopic study revealed coagulation necrosis of liver cells in the periphery of the lobules associated with polymorphonuclear and mononuclear exudate and with fibrin deposition and capillary thrombosis. The process was never associated with hemorrhage. These areas of necrosis were associated invariably with definite inflammatory changes in the stroma of the triads and occasionally there was thrombosis of an arteriole; necrosis of arterioles was not observed and thrombosis of these vessels was uncommon.

The striking feature of the microscopic lesion was the inflamma-

tory reaction in the stroma of the triad. This inflammatory reaction was both acute and chronic and in some instances was present without any demonstrable necrosis of the hepatic parenchyma. Frequently triads were seen in which the reaction was so massive and so acute that the lesion resembled an abscess. The bile ducts themselves contained no exudate and there was no necrosis of biliary epithelium. Parasites were not demonstrable in histological preparations.

Two dogs died of uremia, before either abortion or delivery had occurred, 3 and 5 days following constriction of the renal artery. There was marked elevation of nonprotein nitrogen in both. One of the animals had a moderate elevation of blood pressure and convulsions. Postmortem examination was performed on both dogs but autolysis had rendered the tissues of one useless; in the other no liver lesions were found.

Nine of the 26 dogs gave birth to normal puppies. One died 7 days following delivery and another on the 13th day. The clamps had been applied only 2 and 3 days respectively prior to delivery in these 2 animals. The liver lesions which have been described were not observed in either of these. Of the remaining 7 animals, the clamps were applied at periods ranging from 1 to 7 weeks following the onset of pregnancy in 6 and hypertension had been induced previously in 1. Elevations of blood pressure ranging from moderate to severe were observed. The common finding was an elevation following the application of clamps which declined somewhat at the time of delivery and then rose subsequently. An exception was noted in the dog with persisting induced hypertension, in which the decline in pressure did not take place at the time of delivery but occurred subsequently when it was nursing. When the pups were weaned, the pressure rose again. The response in blood pressure of an animal in which the predelivery rise in pressure was not so great as usual and the postdelivery pressure greater than usual is shown in Chart 1. These dogs did not present symptoms of renal insufficiency or eclampsia at any time.

As stated previously, one animal had a definite elevation in blood pressure (mean pressure 180 mm. Hg.) at the time it became pregnant. The pressure continued to remain elevated and Goldblatt clamps were not applied to the renal arteries. Abortion occurred 5 weeks after the beginning of pregnancy and the blood

pressure at this time declined to 130 mm. of Hg. The dog did not appear to be ill at the time it was killed. Microscopic study of the liver revealed periportal necrosis similar to that already described.

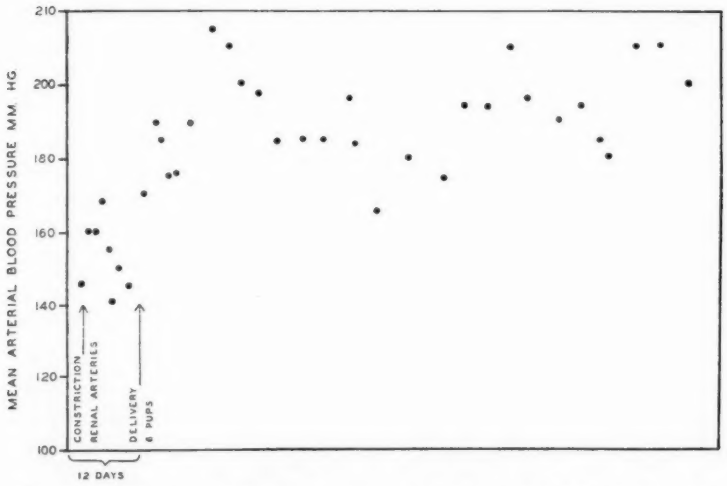


CHART 1. The mean arterial blood pressure before and after delivery in a dog with renal ischemia.

The kidneys of this dog were of considerable interest and because of the infrequent occurrence of natural hypertension in dogs they will be described briefly. Grossly they were approximately normal in size, but they were pale and firm. On superficial examination numerous small clear cysts 1 to 3 mm. in diameter were seen within the capsule. The cortex was slightly narrowed and it was pale and yellowish. The striations were not distinct and they were distorted by irregular streaks or splotches of brownish tissue. The pyramids and the pelves appeared unchanged. The capsule was thickened and because of adhesions between it and the cortex it was stripped away with difficulty. The cysts were seen within the capsule and they overlay small depressions in the cortex. The blood vessels were not thickened.

Microscopic sections showed cystic spaces within the thickened capsule. These spaces were lined by very flat cells and they contained precipitated protein. Surrounding them were accumula-

tions of lymphocytes and plasma cells. This inflammatory reaction in the capsule was continuous with a similar scarring and inflammatory reaction in the cortex. The glomeruli outside of the scarred areas showed syncytial masses of cells with pinkish cytoplasm and relatively large oval nuclei. Most of these masses were near the hilum but some extended far into the glomerulus. These bodies somewhat resembled those described by Goormaghtigh [3] but we have not studied them sufficiently to justify any interpretation of their significance.

Two pregnant animals with normal blood pressure aborted at 5 weeks without constriction of the renal arteries having been induced. These animals did not appear ill at the time they were killed. Examination of one of these dogs revealed no liver lesions. The tissues from the other dog were lost.

For the purpose of comparison the hepatic arteries of two pregnant dogs were occluded. This was done in stages by the method described by Huggins and Post.[4] One of these animals aborted 5 days following the second operative procedure, which was during the fifth week of pregnancy. Liver lesions similar to those already described were found. Normal delivery occurred in the other and no liver lesions were found.

Discussion

One of the most striking features of the experimental disease was that animals which appeared to be ill prior to abortion or delivery did not seem to improve subsequently. Most of these animals died and the evidence indicates that the cause of death was uremia. On the other hand, many of the animals which aborted did not appear to be ill at any time and there was little if any elevation of the nonprotein nitrogen of these animals. The cause for the abortion is not apparent. The incidence of abortion in nonhypertensive pregnant dogs in our laboratory is not known, but several of these animals aborted before the renal arteries were constricted. It is our impression that the incidence is higher than normal in the animals with renal ischemia.

In attempting to explain the contradictory results of Dill and Erickson [2] and of our experiments, it is possible that the former authors, in attempting to cause a marked elevation in blood pressure, produced too severe constriction of the renal arteries. Their

experiments were performed on animals in the later stages of pregnancy and, as has been stated, the blood pressure of a dog with hypertension will decline shortly before and at the time of delivery. Therefore, in order to produce a given rise in blood pressure, it is likely that the renal arterial constriction must be greater in dogs during the terminal stages of pregnancy than in normal nonpregnant animals. At any rate, it is our impression that uremia was the cause of death in most instances.

Dill and Erickson [2] have pointed out certain similarities between the hepatic lesions in hypertensive pregnant dogs and those in human eclampsia. In our studies hepatic lesions were also found, but we feel that these lesions bear only a superficial resemblance to those in human eclampsia. No gross hemorrhages were observed in the livers of dogs in our experiments and hemorrhage was not a feature of the microscopic lesions. The necrosis which occurred in our dogs was of the coagulation type and it was associated with fibrin deposition and fibrin thrombi in the capillaries. Polymorphonuclear leukocytes constituted the greater part of the exudate but some mononuclear phagocytes were also present. These necrotic areas were invariably adjacent to triads and the inflammatory reaction was present in the stroma of the triad as well as in hepatic parenchyma. In addition to acute inflammatory exudate, plasma cells and lymphocytes were also present in the stroma. In most, if not all instances, the lesions in the triad stroma were more conspicuous and older than the lesions in the parenchyma. No inflammatory exudate was seen in the bile ducts and the radicals of the hepatic artery and portal vein also appeared normal. Arteriolar necrosis was not a feature of the lesion.

The genesis of these lesions is unknown but it seems likely that it is different from that in eclampsia because of the absence of hemorrhage and the presence of inflammatory changes in the stroma of the portal triads. Regardless of the differences between these hepatic lesions and those which occur in human eclampsia, our experiments show that hypertension is not a necessary factor in their pathogenesis. This is shown by the occurrence of these hepatic lesions in a dog in which constriction of the hepatic artery had been produced and in which there was no elevation of blood pressure.

REFERENCES

1. Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W. Studies on experimental hypertension. I. The production of persistent elevation of systolic blood pressure by means of renal ischemia. *J. Exper. Med.*, 1934, **59**, 347–379.

2. Dill, L. V., and Erickson, C. C. Eclampsia-like syndrome occurring in pregnant dogs and rabbits following renal artery constriction. *Proc. Soc. Exper. Biol. & Med.*, 1938, **39**, 362–365.

3. Goormaghtigh, N. Une glande endocrine dans la paroi des artérioles rénales. *Bruxelles-méd.*, 1939, **19**, 1541–1549.

4. Huggins, Charles, and Post, Joseph. Experimental subtotal ligation of the arteries supplying the liver. *Arch. Surg.*, 1937, **35**, 878–886.

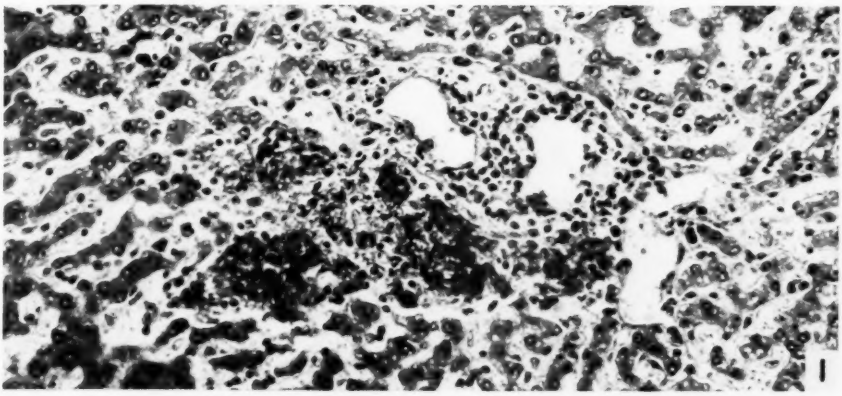
DESCRIPTION OF PLATES

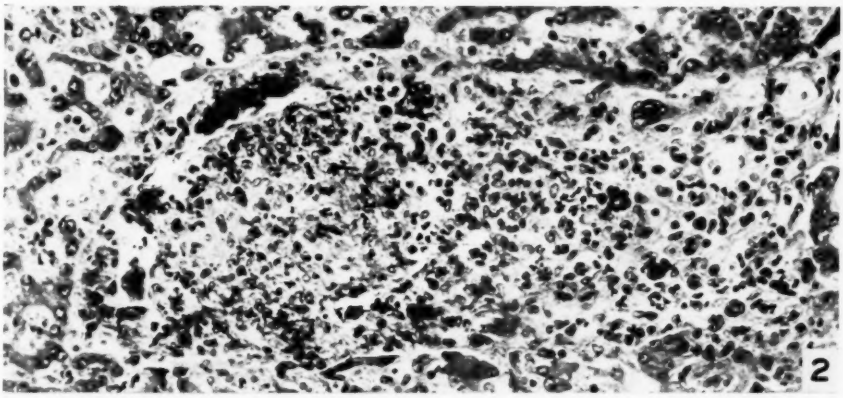
PLATE 7

FIG. 1. Hepatic lesion showing necrosis of liver cells with thrombi in capillaries, and cellular infiltration of the stroma of the triad. Hematoxylin and eosin. × 275.

FIG. 2. Larger lesion showing more extensive cellular infiltration of triad. Necrosis of parenchyma is evident in the lower segment. Hematoxylin and eosin. × 275.

FIG. 3. Still larger lesion in which the cellular infiltration of the triad stroma overshadows the parenchymal lesion. Hematoxylin and eosin. × 200.


1


2

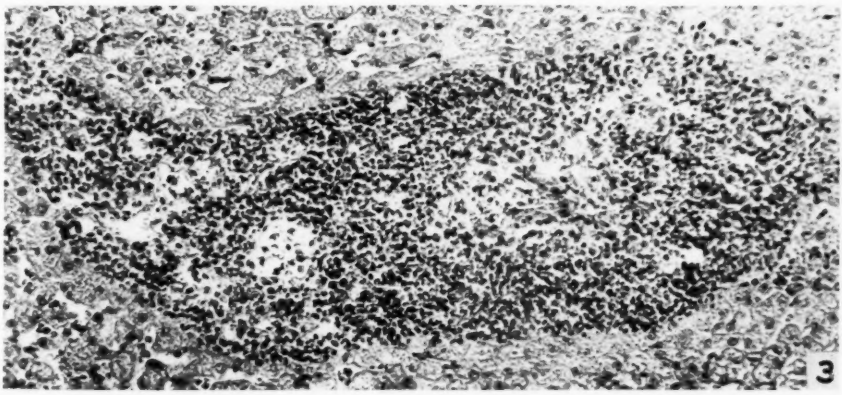

3

PLATE 8

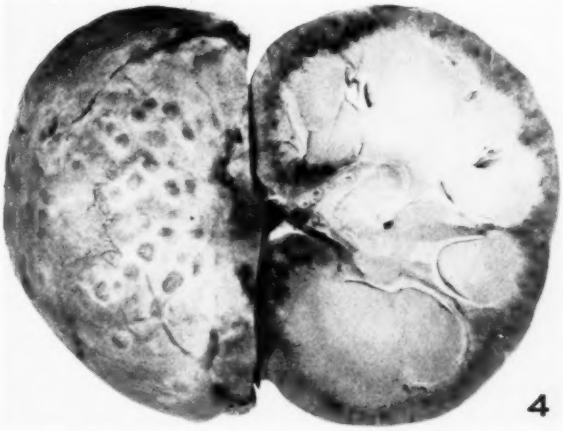
FIG. 4. The kidney of a dog exhibiting a spontaneous hypertension. Note the cysts in the capsule, narrowing of the cortex, and the irregular scars within it.

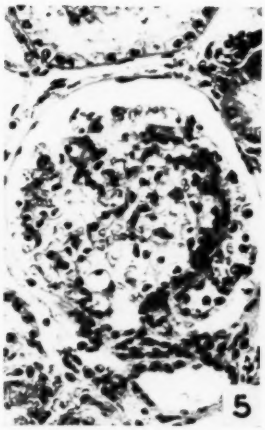
FIG. 5. Glomerulus from this dog showing the extension of the syncytial mass far out into the glomerular tuft. Hematoxylin and eosin. × 225.

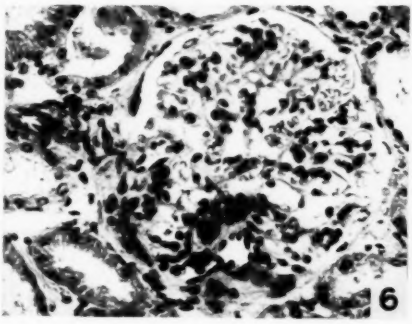
FIG. 6. Similar lesion located at hilum and extending into the glomerulus for a short distance. Hematoxylin and eosin. × 225.

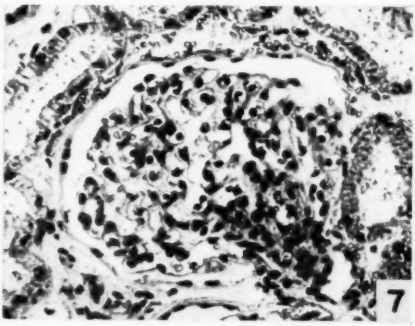
FIG. 7. Lesion like that shown in Figure 6 except that it is confined almost entirely to hilum. Hematoxylin and eosin. × 225.

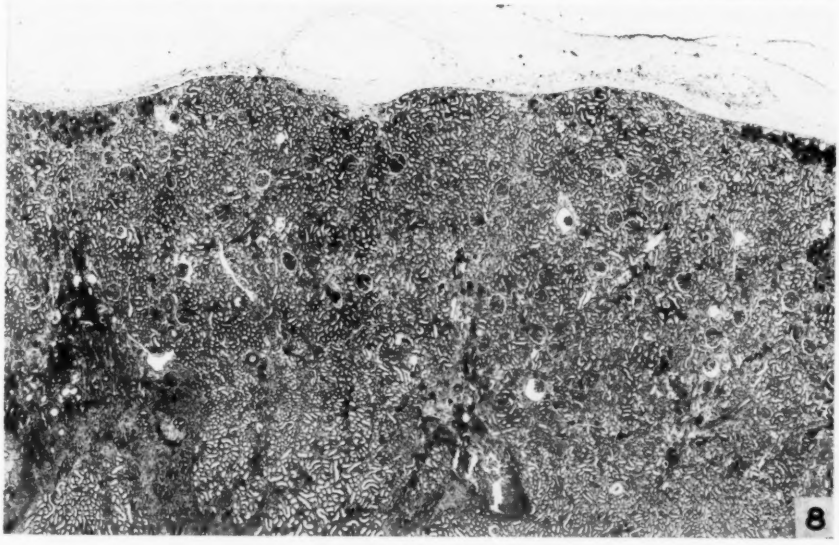
FIG. 8. Low power photomicrograph of kidney cortex of same dog. Note capsular cysts and inflammation of cortical scars. The syncytial masses in some glomeruli may be seen even at this low magnification. Hematoxylin and eosin. × 14.


4


5


6


7


8

STRUCTURE OF THE SMALL CEREBRAL ARTERIES IN HYPERTENSION*

A. B. Baker, M.D.

(From the Division of Nervous and Mental Diseases, University of Minnesota Medical School, Minneapolis, Minn.)

In a previous publication,[1] a study was made of the normal histology of the small cerebral arteries and of the changes that occur in their walls with advancing age. With these observations available for comparison it seemed of interest to study the vascular alterations resulting from hypertension. In reviewing the literature on the structure of small cerebral arteries in hypertensive persons, one is impressed by the paucity of reports. There is a definite lack of agreement as to the exact character of the changes.

Johnson [2] and Ewald [3] described a medial hypertrophy occurring within the arterioles throughout the body. These investigators, however, made no special reference to the cerebral vessels.

Jores [4] recorded a widespread intimal hyalinization occurring within most of the arterioles, excluding those within the skeletal muscles.

Keith, Wagener and Kernohan [5] studied the arterioles in four cases of malignant hypertension. They described a marked intimal hyperplasia with hypertrophy of the internal elastic lamina and of the media. There was some perivascular fibrosis.

Rosenberg [6] reported changes in the brain in seventeen patients suffering from malignant hypertension. He found a thickening of the walls of the arterioles with an associated reduction in the caliber of the lumens. Intimal proliferation occurred but was not constant. The elastica interna was frequently hypertrophied, frayed and reduplicated. The media was increased in thickness.

Moritz and Oldt [7] found the small cerebral arteries altered in but 8 per cent of their cases of hypertension. The changes consisted primarily of a medial hypertrophy and of an endothelial hyperplasia with an increase in the elastic elements.

With these investigations in mind, 53 cases of severe hyperten-

* Prepared on Work Projects Administration Official Project No. 665–71–3–69. Received for publication June 24, 1940.

sion were selected from our autopsy service for study. The diagnosis was substantiated by the blood pressure record, the heart weight, or both. The age groups of these individuals were as follows: 5 cases from 11 to 30 years of age; 5 cases from 31 to 40 years; 13 cases from 41 to 50 years; 17 cases from 51 to 60 years; 4 cases from 61 to 70 years; and 9 cases over 70 years of age. No attempt was made to differentiate between the malignant and the slowly progressive nonmalignant type of hypertension, as was done by Rosenberg.[6] Most of the cases, however, were probably not examples of malignant hypertension, since 31 of the 53 had symptoms lasting more than 2 years prior to death; in 16 the symptoms had been present for 5 years or more. This is in direct contrast to Rosenberg's cases in which the average duration of life after the onset of symptoms varied from 4 to 18 months.

Blocks of tissue were taken from various regions of the brain. These blocks were selected from apparently uninvolved areas. The sections were stained with hematoxylin and eosin, with Weigert's elastic tissue stain, with the Mallory-Heidenhain technic (azocarmine) and with the Bodian stain. As in our previous study,[1] the Mallory-Heidenhain technic has proved invaluable for the study of muscle and fibrous tissue because it demonstrates even the smallest amount of these elements.

Since the structure of the normal small cerebral arteries differs from that of similar-sized vessels elsewhere in the body, it might be of advantage to review their structure briefly at this time. Only arteries varying in size from 50 to 150 μ in diameter will be considered. The internal elastic lamina is both relatively and absolutely thicker than in similar vessels elsewhere in the body. This relative thickness of the elastica tends to persist even into the smallest arteries. The media is composed primarily of a foundation of radially arranged collagenous fibers. This collagenous tissue comprises a surprisingly large portion of this layer and in many cases makes up the major part of the vessel wall. Throughout this connective tissue there are found, in varying numbers, obliquely arranged muscle cells. As the vessel decreases in size, the muscle tissue rapidly disappears and is often difficult to find in vessels under 70 μ in diameter. With increasing age, there results a progressive reduction in the quantity of the elastic and muscular elements of the media. This change is conspicuous and is nearly

complete early in life. When fibrosis is complete it is usually impossible to differentiate the media from the adventitia, the two merging to form a single structure. The adventitial layer of the small cerebral arteries is variable in size. In some cases it is composed only of a few strands of tissue, while in others it is equal in thickness to the adjoining media. As a rule it is composed of a loose network of collagenous fibers.

The cases in the present study were divided into those under 40 years of age and those over this age limit. This particular differential point was chosen because normally only a minimal degree of vascular alteration occurs prior to this age. One can detect normally some medial fibrosis, but most of the intimal and medial changes occur in individuals past the third decade of life.

Age Group 11 to 40 Years

Material from 10 hypertensive patients in the age group 11 to 40 years was studied. In 3, the small cerebral arteries showed extensive changes, while in the remaining 7 they presented little, if any, alteration. A review of the history of the 3 cases showing arterial change revealed the course of the hypertension to have been very rapid. They probably belong to that group described by Keith, Wagener and Kernohan,[5] and Rosenberg[6] as malignant hypertension. Since the alterations of the elements of the vessel wall in these cases were very pronounced, they warrant some detailed description.

Intima. Many vessels showed a marked endothelial proliferation even to the extent of complete vascular occlusion. The usually solid, thick, elastic lamina was irregularly frayed with many tiny fibrils projecting from various portions of the membrane into the adjacent media. Reduplication of the elastica, which does not normally occur until the fifth and sixth decades, was already very extensive. The reduplicated elastic elements occasionally extended inward to narrow or occlude the vessel lumen. Certain segments of the elastica interna showed a definite thickening; other segments were swollen and had lost much of their normal tinctorial properties. These swollen areas occasionally projected inward, producing a definite narrowing of the lumen.

Media. There was a partial to complete reduction of the elastic and muscular elements within the media. In those arteries where

the replacement of the muscle had been complete the fibrosed media merged with the adjacent adventitia, making a separation between them very difficult. The media was much thicker than normal due to the increased fibrous tissue. Normally, a mild fibrosis can be observed in the small arteries of nonhypertensive individuals during the third decade of life; however, it never appears as extensively as in the cerebral vessels of these cases of rapidly progressive hypertension. The medial elements of many of the arteries showed either a diffuse or a patchy hyalinization and loss of their tinctorial properties. These changes seemed to begin in the outer portion of the media and then to spread into entire segments of the vessel wall. Often the hyalinization was complete, replacing all elements (Fig. 1). This hyalinization is very unusual at such an early age since normally it does not occur in the cerebral arteries until the fifth or sixth decades.

Adventitia. The adventitial changes resembled closely those described in the media. In most vessels the adventitia was thicker than normal.

The extreme fibrosis and hyalinization of these small arteries appeared to weaken them to such an extent that erythrocytes often broke through the frayed elastica and fibrosed media and escaped into the perivascular space where they formed a ring-hemorrhage around the vessel.

The 7 remaining cases in this age group were from individuals suffering from chronic hypertension. There was no striking alteration in any of the small cerebral arteries. In an occasional artery there was seen a mild fraying of the elastica interna. Usually, however, this membrane appeared as a deeply staining, thick, compact, laminated structure with only a few regular waves and no signs of reduplication. The media of these vessels was also uninvolved. Although, as is normally the case, the bulk of this layer was composed of collagenous tissue, still the muscular elements were surprisingly prominent. In some of the arteries it appeared that even the normal degree of fibrosis was lacking and the vessels seemed more compact and muscular than is usually the case. Likewise, the adventitia was unchanged. Hyalinization and tinctorial alterations were minimal in this group of cases, although occasionally a mild patchy homogeneity appeared in a few of the collagenous fibers.

From these observations it can be concluded that in this early age group, patients with so-called malignant hypertension present most extensive alterations in the small cerebral arteries, while in the more slowly progressive and chronic cases the arteries are entirely free of visible alteration. In fact, in many vessels in those of the latter group the normal vascular fibrosis is retarded by the hypertensive process.

Age Group 41 to 60 Years

Thirty cases were available for study. In these the findings were much more difficult to evaluate, since normally in this age group extensive changes occur within the small cerebral arteries in the form of reduplication and fraying of the elastica, and fibrosis and hyalinization of the media and adventitia. In none of the hypertensive cases were the alterations in the vessel walls any different from those in normal individuals. Probably the most common observation was a fraying of the elastica interna. The media in most cases contained very little muscle tissue. It was composed almost entirely of collagen and showed a moderate degree of hyalinization. The adventitia was moderately thickened, often frayed and partially hyalinized. Many of these small arteries were surrounded by red cells that had passed through the weakened, frayed elements of the wall and had accumulated within the perivascular spaces.

In a few cases the small arteries were conspicuous by the absence of even those changes normally expected for the age. The elastica was not reduplicated and even its fraying was minimal or entirely absent. Although these vessels were composed predominantly of collagen, throughout this connective tissue there were varying amounts of obliquely arranged muscle cells (Fig. 2). The muscle elements were naturally quite irregular in occurrence but were definitely more prominent than in normal vessels of the same age group. The adventitia was unaltered. Tinctorial changes or hyalinization was not observed. The vessel walls were not thickened and their lumens were not narrowed.

Age Group 61 to 90 Years

Thirteen cases were studied in this group. In these the small arteries showed the same changes as are seen normally at this age.

Reduplication and fraying of the elastica were very pronounced. The media was composed almost entirely of loose bands of collagenous fibres. Curiously enough, in an occasional case even in the eighth decade, the arteries contained a few muscle fibers and cells scattered irregularly through the vessel wall. Their presence was never observed in the vessels of the control series. Tinctorial impairment was very frequent in this group. These tinctorial changes seemed to begin in the center of the vessel wall and gradually spread in all directions to involve large segments of the wall. In some cases only a faint outline of the original vessel could be made out. Hyalinization was also extensive and seemed to follow the same pattern as the tinctorial changes.

In view of the striking absence of definite alterations in the small arteries of these patients with long-standing hypertension, a study was made of the larger cerebral vessels in some of the same cases. Only those were studied in which the smaller vessels were exceptionally free of change. In direct contrast to the smaller arteries, the larger ones (over 300 μ in diameter) showed most extensive alterations, primarily in the form of severe intimal and medial changes. Areolar tissue eventually underwent degeneration with hyalinization. This process produced a marked irregular narrowing of the vessel lumen and in many of the vessels an extensive reduction of the blood flow through the vessel (Fig. 3).

Discussion

In the present study, one is immediately impressed by the paucity of the actual alteration occurring within the small cerebral arteries in cases of long-standing hypertension. In fact, it appears that in certain cases the structure of such vessels in hypertensive patients is spared the routine wear and tear that occurs with increasing age. On the other hand, the larger arteries show definite and often far advanced arteriosclerotic changes with a marked narrowing of their lumens. It is generally believed that protracted hypertension might favor the development of a fairly severe arteriosclerosis, even though in many cases the blood pressure may be elevated for many years without resulting in excessively severe sclerotic changes. In an attempt to explain or correlate the paucity of changes in the smaller arteries and the exten-

sive changes in the larger ones, one could assume that, as the larger vessels become sclerotic and narrowed, there results a reduced blood pressure in the smaller arteries with a corresponding reduction in the wear and tear upon their walls. This might help preserve their normal architecture even in the face of a long-standing hypertension. A phenomenon of this type is not without its parallel in the human body. It has already been shown by Zon [8] that in severe aortic stenosis the base of the aorta shows much less sclerosis or other change associated with age than is normally the case. These aortas have an elasticity corresponding to that of individuals 20 years younger. It was assumed by Zon that the narrowed aortic orifice protected the base of the aorta from the usual wear and tear to which it was exposed.

The present findings may also lend further evidence on the question whether the hypertension or the vascular pathology is the primary event. In view of the above findings it becomes apparent that, as far as the cerebral arteries are concerned, hypertension may exist for years with little or no effect upon the small arteries.

Attention may also be called to the fact that in some of our patients death was due to uremia; correspondingly, definite changes had occurred within the arterioles of the kidney and yet similar changes within the small arteries of the brain were not present. Apparently the arteriolar changes throughout the various organs of the body are not correlated.

Conclusions

1. The average small cerebral artery in cases of long-standing hypertension shows very little structural alteration.

2. The larger arteries often present definite arteriosclerotic changes with a marked narrowing of their lumens.

3. It is possible that the narrowing of the larger vessels reduces the wear and tear upon the smaller ones, thus preserving their normal architecture in the face of the long-standing hypertension.

4. In so-called malignant hypertension, the small arteries show extensive alterations. The elastica interna becomes reduplicated and the media undergoes a rapid fibrosis and patchy hyalinization.

REFERENCES

1. Baker, A. B. Structure of the small cerebral arteries and their changes with age. *Am. J. Path.*, 1937, **13**, 453–461.

2. Johnson, George. Lectures on the pathology, diagnosis and treatment of Bright's disease. Lecture III.—Chronic Bright's disease. *Brit. M. J.*, 1873, **1**, 161–165.

3. Ewald, C. A. Ueber die Veränderungen kleiner Gefässe bei Morbus Brightii und die darauf bezüglichen Theorien. *Virchows Arch. f. path. Anat.*, 1877, **71**, 453–499.

4. Jores, L. Über die Arteriosklerose der kleinen Organarterien und ihre Beziehungen zur Nephritis. *Virchows Arch. f. path. Anat.*, 1904, **178**, 367–406.

5. Keith, Norman M., Wagener, Henry P., and Kernohan, James W. The syndrome of malignant hypertension. *Arch. Int. Med.*, 1928, **41**, 141–188.

6. Rosenberg, Edward F. The brain in malignant hypertension. *Arch. Int. Med.*, 1940, **65**, 545–586.

7. Moritz, Alan R., and Oldt, M. R. Arteriolar sclerosis in hypertensive and nonhypertensive individuals. *Am. J. Path.*, 1937, **13**, 679–728.

8. Zon, L. Unpublished material. (Thesis, University of Minnesota, 1932.)

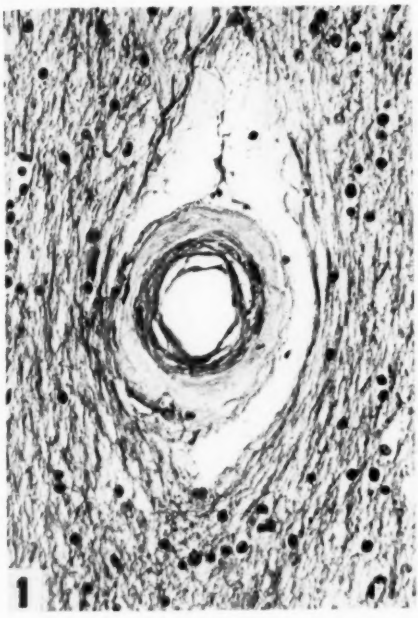
DESCRIPTION OF PLATE

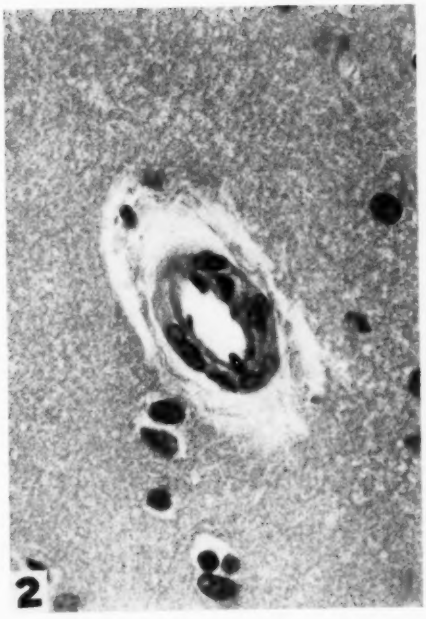
PLATE 9

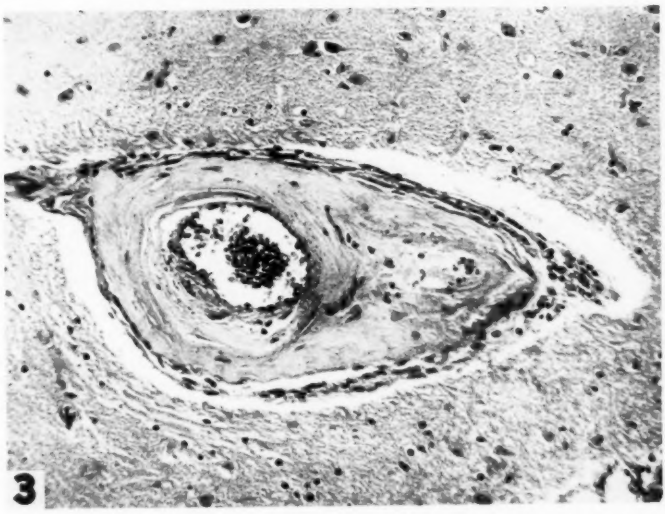
FIG. 1. Hyalinization and tinctorial alterations of a small cerebral artery in a case of malignant hypertension. The hyalinization is complete, replacing all of the wall elements. There is some reduplication and fraying of the intima. Hematoxylin and eosin stain. × 300.

FIG. 2. Structure of a small blood vessel from a chronic hypertensive patient in the fifth decade of life. A few muscle nuclei are still present within the wall elements. Hematoxylin and eosin stain. × 400.

FIG. 3. Structure of a large blood vessel from a chronic hypertensive patient. Note the severe degree of sclerosis. The vessel lumen is greatly reduced in size. Hematoxylin and eosin stain. × 200.


1


2


3

NECROSIS OF THE BONE MARROW WITH FAT EMBOLISM IN SICKLE CELL ANEMIA*

LEO J. WADE, M.D., AND LEWIS D. STEVENSON, M.D.

(From the Department of Pathology, Cornell University Medical College, and the New York Hospital, New York, N. Y.)

INTRODUCTION

Fat embolism has been described in a wide variety of clinical conditions. The importance of this complication in instances of trauma to bone or fatty tissues is well recognized. Few have agreed, however, that the finding of fat emboli in other conditions is of any practical importance.

A number of investigators have shown that the examination of routine necropsy material for fat embolism will reveal positive findings in a certain proportion of the cases. Lehman and McNattin [1] found varying degrees of fat embolism in the lungs in 37 of 50 autopsies. The embolism was described as moderate to marked in 13 instances, 6 of which were unassociated with trauma. More recently, Vance,[2] in a study of 246 necropsies, found "very slight fat embolism" in only 7 of the 82 cases which were unassociated with trauma. His conclusions were in accordance with the much earlier observations of Warthin,[3] who stated that in nontraumatic cases "the fat is so small in amount and the lesions so few, as to be of pathologic interest only."

We have recently had opportunity to study a case of sickle cell anemia in which the clinical picture and the pathological findings leave no doubt that fat embolism was an important factor in bringing about the patient's death. Groskloss [4] and Warthin [3] stated that fat embolism occurs in certain anemias. We have been unable, however, to find any report of a case similar to ours.

REPORT OF CASE

Clinical History. (N. Y. H. No. 59202.) The patient was a Greek housewife, 49 years old, who was admitted to the New York Hospital on four occasions.

Family History. The available family history was meager, but both of the patient's parents were said to be dead of causes unknown. They were born

* Received for publication May 27, 1940.

in Greece, of Greek parentage. There was no known history of familial disease.

Past History. The patient's general health was said to have been good, except for "nervousness," until March 1934, when she was admitted to the Surgical Service. She had been awakened from her sleep by a severe pain said to have been present throughout her entire body, but most severe in the right upper quadrant of the abdomen. The onset of the pain was associated with vomiting; attempts to drink water or to take food were followed by further vomiting. The pain was constant in nature and unrelieved by heat. The patient's husband stated that she had had a similar attack 3 years before, but none before or after to his knowledge. The only positive physical findings were: anemia, slight icterus, slight spasm and definite tenderness in the right upper quadrant.

A tentative diagnosis of acute cholecystitis was made and a laparotomy was performed. No stones were present and the gallbladder was not inflamed. No lesion was found to explain the patient's symptoms. The patient received four transfusions with a total of 1450 cc. of blood. She was discharged on April 15, 1934.

The patient was brought to the Emergency Pavilion on June 9, 1934, complaining of pains "like pins and needles" over the entire body. The patient was highly excitable but the physical examination was otherwise negative. She was given phenobarbital and sent home.

The patient was admitted to the Gynecological Service on January 22, 1935 because of metrorrhagia. Dilatation and curettage were done. The endometrial tissue removed was that of a postmenopausal uterus.

On October 5, 1935 the patient was again seen in the Emergency Pavilion. She complained of severe pain "just like last time," associated with nausea and vomiting. The patient was moaning, screaming, and lashing about in bed. She was admitted to the Surgical Service where she was observed for 24 hours. The pain subsided and in view of an entirely negative physical examination the patient was discharged.

The patient got along well until 1939. In that year she had several episodes of pain, nausea and vomiting, associated with "darkening of the skin."

Present Illness. At 2 a.m. on January 1, 1940 the patient was awakened from her sleep and caused to cry out by excruciating pain in the lumbar region of the spine. The pain returned in paroxysms and became generalized. The paroxysms of pain caused the patient to "break out in cold sweat." The pain was associated with vomiting and the patient continued to vomit all ingested food or water up to the time of admission to the Medical Service on January 4. On the day before admission the patient had severe shaking chills; her temperature rose to 102° F. and she became comatose.

Physical Examination. Temperature 39° C. Pulse 122. Respirations 38. Blood pressure 132/64. The patient was obviously critically ill. She was semicomatose. The neck was slightly stiff. There were petechiae in the conjunctivae and skin. The spleen was palpable. The liver was slightly enlarged. There was questionable icterus.

Laboratory Findings. These are summarized in Table I.

Course and Treatment. Throughout the patient's hospital stay her temperature varied from 38.6° to 40° C. The patient was at all times semistuporous. She was given six blood transfusions, totalling 2250 cc. of

TABLE I
Laboratory Findings in Case Reported

	Admission												
	I (1934)					II (1935)	III (1935)	IV (1940)					
Date	3/19	3/23	3/24	3/26	3/29	1/22	10/5	1/4	1/6	1/8	1/9	1/12	1/13
R. b. c. (millions per cu. mm.)	2.7	1.9	2.4	3.2	3.6			2.3	3.1	3.7	3.4	3.5	3.6
Hb. (14.5 gm. per 100 cc. = 100%)	55%	38%	48%	60%	70%	74%	85%	41%	50%	60%	60%	60%	66%
Cell volume						33%		15%			23%		
Sickle cells			+	+	—				—				
Reticulocytes									4.2				
Anisocytosis			+	+				+	+	+	+	+	+
Poikilocytosis			+	+				+	+	+	+	+	+
Nucleated r. b. c. (% of w. b. c.)			50%	67%				6%		35%	31%	22%	15%
W. b. c. (corrected)	8950	23,300	23,000	20,000	11,800	3250	7200	11,700	12,000	8500	7100	6500	7600
Adult polys	26%			23%				50%	60%	57%	55%	34%	21%
Immature polys	52%			56%				15%	18%	9%	11%	43%	47%
Lymphocytes	15%			11%				29%	20%	23%	27%	22%	30%
Platelets				260,000									
Bleeding time*				3 min.					3.5 min.				
Clotting time**				4 min.					9 min.				
Fragility					Normal				Normal				
Sedimentation rate†						0.1		0.05			0.1		
Icteric index	19	36	25	23	19			15		9			

* Duke method: Normal = 1 to 3 minutes
** Lee and White: Normal = 5 to 10 minutes
† Rourke and Ernstene: Normal = 0.08 to 0.35 mm. per minute

citrated blood. She was unable to take food by mouth and was given repeated infusions of 5 per cent glucose in saline solution. The petechiae noted on admission gradually faded and no new ones appeared. Her neck remained stiff but no Kernig or Babinski signs were elicited. The spinal fluid pressure was 140 mm. of water, the fluid was clear and there were three lymphocytes per cu. mm. Cultures of the fluid were sterile. The protein was 40 mg. per cent; sugar 92 mg. per cent; chlorides 740 mg. per cent; and the Wassermann negative. The patient's condition remained unchanged until the tenth hospital day, when she became more deeply comatose and her respirations became rapid and shallow. The blood pressure fell to 90/60. She became deeply cyanotic. The patient was placed in an oxygen tent and given respiratory stimulants but these were of no avail and the patient expired a few hours later.

Postmortem Examination

The description will be confined to the positive findings.

Macroscopic Examination. The *spleen* weighed 350 gm. It was adherent to the adjoining structures but was easily separated. The capsule was pale green in color and slightly wrinkled. On the surface were many gray areas with irregular "map-like" boundaries. These varied from a few millimeters to 2 cm. in diameter. The spleen was moderately firm but definitely "lumpy" in consistency. The "lumpy" areas of increased density corresponded to the gray areas described above. The spleen cut with a gritting sensation. The cut surface was dark red in color except for gray areas similar to those described on the surface. These occupied approximately 25 per cent of the cut surface. The *liver* weighed 1610 gm. Many pale, roughly circular areas, less than 1 mm. in diameter, were apparent on the cut surface of the liver. The *kidneys* each weighed 160 gm. The glomeruli stood out prominently as tiny hemorrhagic spots on the capsular and cut surfaces. The *bone marrow* was pale in color. The *arachnoid* of the interpeduncular space was slightly thickened. There was widespread cortical atrophy of the *brain.*

Microscopic Examination. The capsule and trabeculae of the *spleen* were moderately thickened by collagen fibers, between which were clusters of golden brown refractile bodies, roughly cylindrical in outline and in many instances segmented so as to resemble "bamboo poles." These bodies were made up chiefly of iron pigment. Similar masses of iron pigment and collagen fibers were present throughout the pulp. Such nodules were surrounded by dilated sinusoids containing many sickle cells and macro-

phages. The macrophages were loaded with erythrocytes and iron pigment. All of the malpighian corpuscles appeared to be involved. There were pale-staining areas of necrosis scattered irregularly through the pulp. The blood vessels were surrounded by collagen fibers and iron pigment but no thrombi were found.

The sinusoids of the *liver* were congested. The adjacent liver cells were extensively vacuolated and contained an unusually large amount of bile pigment. There were many bile thrombi in the biliary canaliculi. Small focal areas of necrosis of liver cells were present.

Material obtained by aspiration biopsy of the *sternal marrow* before death was necrotic and could not be stained satisfactorily. Sections prepared from postmortem material were also characterized by widespread necrosis with marked reduction in the blood-forming constituents and fat. The necrotic areas consisted of a faintly eosinophilic network in which were scattered granular debris, occasional polymorphonuclear leukocytes and numerous macrophages loaded with fat and chromatin particles. Foci of erythrogenesis contained mature cells, many of which were sickle shaped. Myelogenesis was normal. The megakaryocytes were reduced in number.

In the *brain* were many small areas of focal necrosis, both in the gray matter and in the white matter (Fig. 1). In the middle of some of these foci a few red blood cells were present. In addition there were many small hemorrhages, both in the cerebral cortex and in the cerebellum. These hemorrhages were usually perivascular. In these areas many of the red blood cells showed extreme degrees of sickling (Fig. 2). Wherever hemorrhage had occurred there was considerable proliferation of microglia cells although none had yet attained the form of gitter cells. Associated with this was a definite increase in the number of astrocytes in these areas. There were many fat emboli throughout the brain and in many instances the small areas of focal necrosis showed a capillary, either in the center of the area or just to one side, filled with droplets of fat (Fig. 3).

Frozen sections of lung, liver, kidney and spleen stained for fat contained many droplets in the arterioles and capillaries (Figs. 4 and 5).

Anatomical Diagnosis. Sickle cell anemia; splenomegaly (350

gm.) with areas of necrosis and siderofibrotic nodules; icterus; bile pigment thrombi in biliary canaliculi; focal necrosis of the bone marrow; fat emboli in lungs, brain, liver, spleen and kidneys; disseminated focal necrosis of the brain due to fat emboli.

Discussion

Sickle cell anemia is rare in the white race and it is quite unusual for patients more than 30 years of age to present clinical evidences of this disease. The diagnosis of sickle cell anemia in this instance, however, is based upon clear-cut clinical and anatomical findings. The history of repeated bouts of pain, both abdominal and muscular, with vomiting and "darkening of the skin," is typical. These were associated with a normocytic anemia, leukocytosis, mild icterus, large numbers of nucleated red blood cells, and a normal bleeding and clotting time. The sickling phenomenon was so prominent as to be manifest even in routine blood smears on several occasions. The anatomical changes in the spleen were precisely those described by Diggs.[5] The finding of large numbers of sickle cells in the sections further corroborates the diagnosis.

There are certain respects, however, in which this case differs from previously described cases of sickle cell anemia. The clinical history suggests that the terminal attack began in the same manner as the previous ones. On the third day, however, the patient developed chills and fever, became comatose, and presented numerous petechiae over the skin and in the conjunctivae. The petechiae faded and no new ones appeared but the patient remained comatose. It was on the third day, no doubt, that the blood was "showered" with fat emboli. The signs and symptoms a few hours before death suggest a second "shower" of emboli. The clinical picture is explained thereby and the postmortem findings are compatible with this interpretation.

The source of these fat emboli is not entirely clear. The bone marrow was necrotic, as has been previously described in cases of sickle cell anemia,[6,7] and it seems probable that this was the source of the emboli. In recorded instances of fat embolism, necrotic purulent foci and septic processes of the bone marrow have been described as the source of fat emboli by some of the early observers.[8] Lehman and Moore,[9] on the basis of *in vitro*

experiments, concluded that fat embolism might be produced readily in the bone marrow on a nontraumatic basis purely by the absorption of histamine from injured tissue into the blood stream.

The central nervous system involvement in this case is of particular interest. Several observers have previously described central nervous system lesions. Indeed, Bridgers [10] has pointed out that signs and symptoms of cerebral vascular thrombosis or intracranial hemorrhage may be the first manifestations of sickle cell anemia. Sensory and motor disturbances, headaches, nausea and vomiting, and signs of meningeal irritation are frequently reported.[11] The lesions of the nervous system have been inadequately described in most instances. Bridgers [10] described obliterative vascular lesions in one case. In another he reported the finding of multiple focal areas of necrosis and hemorrhage, apparently similar to those described in our case. The clinical picture in the two cases is also very similar.

The possible rôle of trauma or some toxic agent has been studied carefully. No evidences that either factor was involved could be obtained from careful questioning of the patient's family, or from the necropsy findings. Inasmuch as the clinical picture was fully developed prior to the patient's admission to the hospital, and in view of the glial proliferation found in the central nervous lesions, it is improbable that any manipulations such as venepunctures or subcutaneous injections following admission to the hospital played any important rôle in producing the lesions described. The similarity of the complaints and of the laboratory findings on each of the several admissions makes it improbable that toxic damage to the bone marrow by some extraneous substance need be considered.

Summary

A case of sickle cell anemia in a Greek housewife, 49 years old, is described. The known clinical history of acute exacerbations is of 6 years' duration. The terminal episode is characterized chiefly by cerebral manifestations which are adequately explained by the presence of widespread focal areas of hemorrhage and necrosis in the nervous system. These result from fat emboli which we believe to be secondary to necrosis of the bone marrow.

REFERENCES

1. Lehman, Edwin P., and McNattin, Robert F. Fat embolism. II. Incidence at postmortem. *Arch. Surg.*, 1928, **17**, 179–189.

2. Vance, B. M. The significance of fat embolism. *Arch. Surg.*, 1931, **23**, 426–465.

3. Warthin, Aldred Scott. Traumatic lipaemia and fatty embolism. *Internat. Clin.*, 23rd series, 1913, **4**, 171–227.

4. Groskloss, Howard H. Fat embolism. *Yale J. Biol. & Med.*, 1935–36, **8**, 59–91, 175–197, 297–316.

5. Diggs, L. W. Siderofibrosis of the spleen in sickle cell anemia. *J.A.M.A.*, 1935, **104**, 538–541.

6. Diggs, L. W., Pulliam, H. N., and King, J. C. The bone changes in sickle cell anemia. *Southern M. J.*, 1937, **30**, 249–259.

7. Graham, George S. A case of sickle cell anemia with necropsy. *Arch. Int. Med.*, 1924, **34**, 778–800.

8. Scriba, J. Untersuchungen über die Fettembolie. *Deutsche Ztschr. f. Chir.*, 1880, **12**, 118–220.

9. Lehman, Edwin P., and Moore, Robert M. Fat embolism, including experimental production without trauma. *Arch. Surg.*, 1927, **14**, 621–662.

10. Bridgers, William H. Cerebral vascular disease accompanying sickle cell anemia. *Am. J. Path.*, 1939, **15**, 353–362.

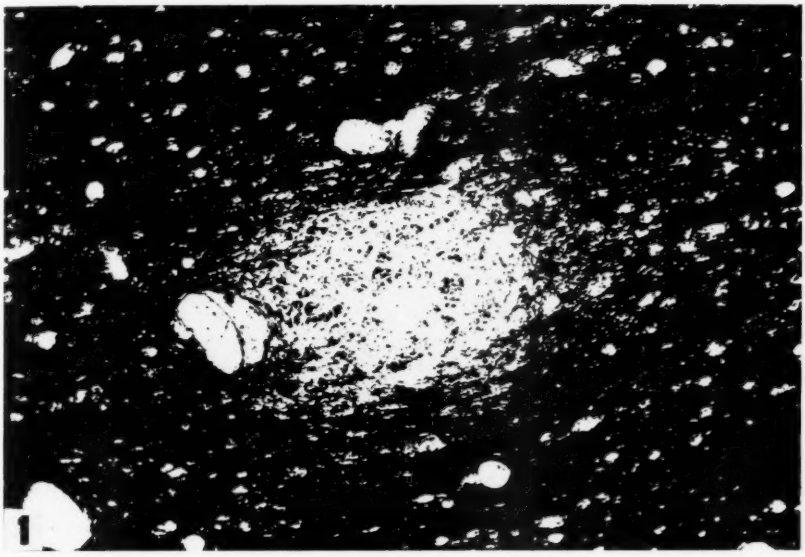
11. Diggs, L. W., and Ching, R. E. Pathology of sickle cell anemia. *Southern M. J.*, 1934, **27**, 839–845.

DESCRIPTION OF PLATES

PLATE 10

FIG. 1. Section of cerebral cortex (Loyez' stain for myelin sheaths) showing focal necrosis of the white matter. × 190.

FIG. 2. Hemorrhage near wall of the third ventricle (Loyez' stain for myelin sheaths) showing sickle cells. × 1500.



Wade and Stevenson Fat Embolism in Sickle Cell Anemia

PLATE 11

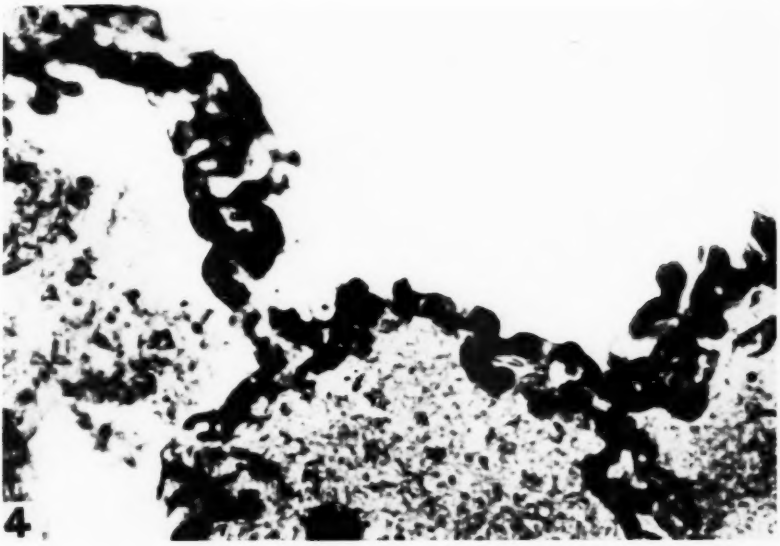
FIG. 3. Section of superior frontal gyrus of brain (Marchi's stain) showing fat emboli in capillaries surrounded by area of necrosis. × 360.

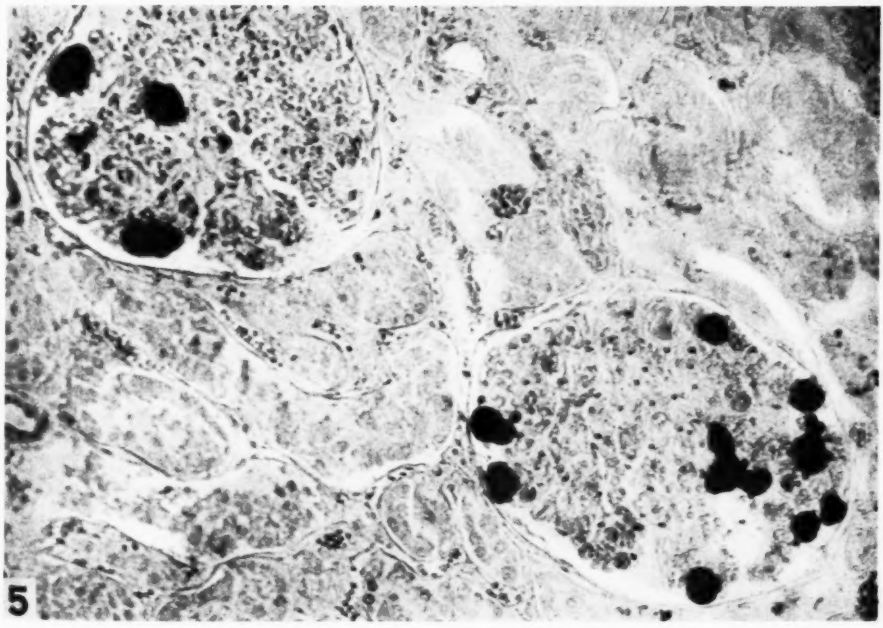
Wade and Stevenson Fat Embolism in Sickle Cell Anemia

PLATE 12

FIG. 4. Section of lung (Sudan III stain) showing fat emboli in the capillaries. × 650.

FIG. 5. Section of kidney (osmic acid stain) showing fat emboli in the glomerular capillary loops. × 650.


4


5

ISOLATION OF THE VIRUS OF HERPES SIMPLEX AND THE DEMONSTRATION OF INTRANUCLEAR INCLUSIONS IN A CASE OF ACUTE ENCEPHALITIS*

MARGARET G. SMITH, M.D., EDWIN H. LENNETTE, M.D., AND HAROLD R. REAMES, Ph.D.

(From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.)

Although there is an extensive literature pertaining to the virus of herpes simplex as an etiological agent of encephalitis in man, there is no reported case of fatal encephalitis from which the herpes virus has been isolated which has shown intranuclear inclusions in the brain resembling those of herpetic lesions. In the first report of the Mathewson Commission,[1] a summary of the literature concerning the relation of the herpes virus to encephalitis mentioned only 9 instances of the isolation of a virus identified as that of herpes simplex from cases of encephalitis. In 5 of these, the virus was isolated from the brain; in 3, from the spinal fluid; and in 1, from the nasopharynx.

Subsequent to this report, Gay and Holden [2] isolated a virus from the brain of a man who died during an acute exacerbation of chronic encephalitis. They considered it identical with the herpes virus. A second virus, isolated by Gay and Holden [2] from the brain of a laboratory worker, 27 years old, who died following the bite of a monkey, was likewise reported as the virus of herpes simplex. However, it seems that the latter virus, also isolated and described by Sabin,[3] was not the virus of herpes simplex but that now designated as virus B. Also, with brain material from 3 cases of encephalitis, all occurring in children following measles, Gay and Holden [2] produced in rabbits, skin lesions which were in keeping with those of herpes. In none of these 3 cases was the virus identified as that of herpes.

Other investigators [4-8] have reported the presence of an agent which they considered the virus of herpes, although not definitely established as such, in the brain or in the spinal fluid of patients

* This work was supported by a grant from the St. Louis Encephalitis Committee.

Received for publication June 4, 1940.

with symptoms of encephalitis or meningitis. The methods used in these investigations to demonstrate the presence of herpes virus were the production of an encephalitis in rabbits following the intra-ocular or intracerebral inoculation, or the production of corneal lesions alone following intra-ocular inoculation. The accuracy of the latter method has been criticized by Rupilius and Szekely,[9] who questioned the criteria used for judging a positive corneal reaction for herpes virus. They were able to produce a punctate keratitis, similar to that described by some investigators as a positive reaction for the herpes virus, by inoculation of non-specific solutions.

In addition, the etiological significance of the herpes virus, when found in the brain or spinal fluid, has been questioned because of its occasional demonstration in the spinal fluid of individuals showing no evidence of encephalitis or herpetic eruptions. Such instances, however, are less frequent than is commonly supposed, and according to Doerr and Hallauer [10] latent infections of the central nervous system with herpes virus never have been proven. In 1937 Zurukzoglu [11] summarized the reports of results obtained by a number of investigators with spinal fluid from individuals showing no evidence of encephalitis or herpetic eruptions. In five different investigations, in which a total of 108 spinal fluids were tested for the presence of herpes virus, the results were entirely negative. Zurukzoglu cited the following investigations in which positive results were obtained. The herpes virus was isolated from only 1 of 100 spinal fluids examined by Flexner and Amos. Of 50 fluids examined by Zurukzoglu, 2 gave positive results. Zurukzoglu also tested the spinal fluid from 8 cases, without clinical symptoms of encephalitis, shortly after the occurrence of herpes labialis. Two of these gave evidence, by intra-ocular inoculation of rabbits, of herpes virus in the spinal fluid. The only results reviewed, which differed strikingly from the others, were those of Bastai and Busacca who reported the presence of the herpes virus, demonstrated by intra-ocular inoculation of rabbits, in 18 of 22 spinal fluids from unselected cases.

In addition to these reports of the demonstration of a virus in the brain or spinal fluid, there are a number of reports [12,13] of the demonstration of a virus which was capable of producing keratitis and/or encephalitis by corneal inoculation of rabbits in

the nasopharynx or saliva of patients with clinical symptoms of acute or chronic encephalitis. The etiological significance for encephalitis of the herpes virus thus obtained may be questioned in view of the frequent occurrence of herpes infections and also in view of the demonstration of the herpes virus in the saliva of normal individuals.[14]

In 1933 Dawson [15] reported a case of fatal lethargic encephalitis in a boy 16 years of age. Sections of the brain showed, in addition to minute hemorrhages and perivascular infiltration of lymphocytes, intranuclear inclusion bodies in the cerebral lesions comparable to those seen in herpetic infections. In this instance no etiological agent was isolated. To our knowledge this is the only description in the literature of intracellular changes in the brain lesions of human encephalitis suggestive of those produced by the herpes virus. However, these lesions were believed not to be identical with those of herpes simplex because the nuclear inclusions were never of the very large granular type seen in herpes.

It is the purpose of this paper to present a case of encephalitis occurring in an infant 4 weeks old in which intranuclear inclusion bodies, compatible with those of herpes, were found in the cerebral lesions and from which a virus, identical with that of herpes simplex, was isolated from brain tissue. Because of the importance of establishing the identity of the etiological agent, a detailed account of its investigation is given.

Report of Case

Clinical History and Observations. The patient, a white male infant 4 weeks old, was admitted to St. Louis Children's Hospital because of irritability, refusal to nurse, and twitching of the left side of the body.

No family history of significance was obtained. The infant was born at home 1 month prematurely; the delivery was normal. Until 4 days before entry to the hospital he had been breast fed, had developed normally and appeared in good health. At that time, however, it was noticed that he was irritable and fretful, but there was no noticeable fever and no vomiting. Two days before admission to the hospital he began to complain when moved and to scream out suddenly. On the day before admission, twitching of the left arm and leg was first noticed and the child cried continuously. On the day of admission the twitching of the left side of the body continued, the child refused to nurse and had a slight fever.

The patient appeared moderately well nourished; he was listless and was having occasional twitchings of the left arm and leg. The fontanelle was full

and tense. Both ear drums were dull, and the left one bulging. To external examination the eyes were normal except for a slight clouding of the cornea. Examination of the eye grounds disclosed a pale zone about the optic disks; however, a diagnosis of optic atrophy was considered questionable. The tongue and mucous membranes of the mouth appeared normal. The pharynx was not hyperemic. A seborrheic dermatitis was present on the scalp, but no other abnormalities of the skin were observed. The white blood cell count was 18,000; the red blood cell count, 4,100,000; and the hemoglobin content of the blood, 11 gm. per 100 cc. The temperature on admission was only slightly elevated and later dropped to subnormal levels.

As a result of the first lumbar puncture, bloody, sterile fluid was obtained. Several other attempts were made to obtain spinal fluid from the cisterna and from the ventricles, and a small amount of blood-tinged cisternal fluid was obtained on one occasion.

The child's illness steadily progressed. The fontanelle became more and more tense; the convulsive movements of the extremities continued; and there was a generalized convulsion on the day after admission to the hospital. Death occurred on the fifth hospital day. A clinical diagnosis of acute encephalitis was made.

Postmortem Examination

A complete autopsy revealed no macroscopic abnormalities other than those in the brain. On gross examination of the brain the leptomeninges appeared hyperemic; and in the region where the intraventricular punctures had been made there were a few small clots of blood, but no extensive hemorrhage. Material was taken from the frontal cortex for animal inoculation. Nothing unusual was observed when a cut was made through this portion of the brain; however, the entire brain was unusually soft, even for that of a young infant. Unfortunately, only the cerebellum and the brain stem were saved for microscopic study.

The microscopic study of organs other than the brain revealed nothing abnormal.

Sections from the pons, medulla, and cerebellum were stained with hematoxylin and eosin, phloxine-methylene blue, and with bacterial stains. There was an extensive inflammatory process involving the brain tissue and, to some extent, the meninges. In certain areas the meninges were entirely normal, while in others, always in association with changes in the underlying brain tissue, there was an infiltration of cells concentrated about blood vessels. These cells were chiefly lymphocytes and larger mononuclear cells. Only an occasional polymorphonuclear leukocyte was seen. The walls of the meningeal blood vessels were not damaged.

In general, the changes seen in the sections from the three parts of the brain were alike, but less severe in the sections from the cerebellum. Perivascular infiltration of cells like that occurring in the meninges was conspicuous within the brain substance. The vessel walls, however, appeared normal except that the lining endothelial cells were often unusually large. There were focal accumulations of cells having either round or elongated nuclei: some with indistinctly outlined cytoplasm, others with sharply outlined cell margins (Fig. 1). These focal accumulations of cells closely resembled the focal lesions, largely of microglial origin, seen in other types of virus encephalitis. In some instances they were associated with degenerating nerve cells. In other microscopic fields a more diffuse inflammatory reaction of mononuclear cells occurred, involving, in some instances, groups of large ganglion cells which showed stages of degeneration. Isolated degenerating nerve cells surrounded by a cluster of small mononuclear cells were also seen. In sections from both the pons and medulla, areas of necrosis were present which were slightly larger than a low power field (16 mm. objective). This necrosis was not localized about vessels. In fact, the vessels were remarkably well preserved, even in the necrotic zones. One necrotic area had undergone liquefaction (Fig. 2); in the others there were many large fat-holding phagocytes and remnants of necrotic brain tissue.

In the cerebellum the Purkinje cells appeared normal except in localized areas where they, together with the adjacent small nerve cells, were undergoing necrosis.

Intranuclear Inclusions. The degenerative changes in individual nerve cells were especially interesting. Some of these cells showed pyknotic nuclei and deeply stained, shrunken cell bodies; others were poorly outlined and showed varying degrees of karyolysis. Many cells, however, showed more specific nuclear changes in the form of intranuclear inclusions which varied somewhat in appearance. In some nuclei the chromatin was marginated at the nuclear membrane about an acidophilic central body (Figs. 3 and 4). The smaller of these central bodies were four to five times the usual size of a nucleolus. Occasionally, there was a fine network of light blue-staining material which radiated from the acidophilic central body to the basophilic margin. In other

cells a clear area surrounded the acidophilic inclusion and at times a small, basophilic nucleolus was seen in addition to the marginated chromatin. Many of the inclusions conformed in shape to the general contour of the nucleus which contained them. Other nuclei had a different appearance. In these there was a very deeply stained margin of chromatin arranged in dots, while the rest of the nucleus was completely occupied by a material which stained lilac with phloxine-methylene blue and was often definitely granular (Figs. 5 and 6). Nuclear changes of both types were interpreted as intranuclear inclusion bodies corresponding to forms seen in known herpetic lesions.

Isolation of the Virus

A piece of cortex was ground without abrasive in a small amount of nutrient broth. After light centrifugation, 0.03 cc. of the supernatant fluid was inoculated intracerebrally into 6 Swiss mice. On the third day after inoculation, 4 of the 6 mice were found dead and the remaining 2 were observed in convulsions. The brains of these mice were removed aseptically, cultured, and passed to other Swiss mice, each of which received 0.03 cc. of a 10 per cent dilution of the brain material; these animals died or were killed, after being observed in convulsions, on the third day following inoculation. The infective agent has been maintained in Swiss mice until the present, and the brains of mice have been used as the source of material for studying its characteristics.

To determine the nature of the infectious agent, aerobic cultures of the human brain were made in dextrose infusion broth and both aerobic and anaerobic cultures of the infected mouse brains were made in broth and on blood agar. No organisms were grown. Sections from the human brain and from the brains of experimental mice, stained by the MacCallum-Goodpasture method, were studied for bacteria. None could be demonstrated. The infectious agent was therefore considered to be a virus and, as a matter of convenience, was designated "R. T." virus.

Infected mouse brains from early passages were stored in 50 per cent glycerin in Locke's solution at 5° C. After 5½ months the virus from the second mouse brain passage remained active, apparently retaining its original infectivity; a 10 per cent emul-

sion inoculated intracerebrally killed mice in slightly less than 3 days.

The filtrability of the virus was tested as follows. A 10 per cent suspension of infected mouse brain was made in 2 per cent normal horse serum broth, centrifuged at 2000 r.p.m. for 5 minutes in a horizontal centrifuge, and the supernatant fluid further diluted to 1 per cent with horse serum broth. This suspension was put through two new Berkefeld N candles, prepared, after washing, by filtering 30 cc. of 2 per cent normal horse serum broth through each. Neither filtrate was infectious for mice by intracerebral inoculation, while the unfiltered 1 per cent brain suspension was still infectious when diluted tenfold, all of 4 Swiss mice dying following intracerebral inoculation.

On a number of occasions, when the same procedure was carried out using nutrient broth as a diluent and for preparing Berkefeld N and V filters, no virus was demonstrated in the filtrates. These results are not surprising in view of the known difficulty in the filtration of herpes virus.

Response of Laboratory Animals to the Virus

Mice. As already stated, mice succumbed in 3 days to intracerebral inoculation of 10 per cent emulsions of infected mouse brain; and the virus, maintained by mouse passage, was uniformly infectious by this route in dilutions as great as 10^{-4}. Following subcutaneous inoculation of as much as 0.25 cc. of a 10 per cent emulsion of infected mouse brain, an occasional mouse developed paralysis, but the great majority remained well.

After intracerebral inoculations of the virus the mice frequently showed little or no evidence of illness until they began to have active muscular twitchings or generalized convulsions. Death in severe convulsions usually followed shortly thereafter.

Lesions were not found in organs other than the central nervous system. In sections of the brain the most conspicuous lesion was a meningeal exudate extending into perivascular spaces within the brain. In some areas the exudate was composed of well preserved, small lymphocytes and slightly larger mononuclear cells. Other fields showed a partially necrotic meningeal exudate, in the debris of which lymphocytes and mononuclear cells, together with polymorphonuclear leukocytes, were still recogniz-

able. In the mouse brain, inclusions in the nerve cells have been but rarely observed.

Guinea Pigs. Of 8 guinea pigs inoculated intracerebrally with 0.15 cc. of the supernatant fluid of a 10 per cent emulsion of infected mouse brain, 6 remained well and 2 died. One of the latter, which died on the eighth day following inoculation, was autopsied. Microscopic sections of the brain showed a slight cellular infiltration in the meninges and perivascular spaces similar to that seen in the less severe reactions in the mouse brains. No intracellular inclusions were observed in several sections.

The corneae of 2 guinea pigs were scarified and rubbed with a 10 per cent emulsion of infected mouse brain. In each animal, beginning on the fourth day, a slight opacity of the cornea appeared. This opacity decreased after the seventh day, and neither animal showed other symptoms.

Three guinea pigs, inoculated intradermally with 0.2 cc. of a 10 per cent emulsion of infected mouse brain, developed, on the fifth day thereafter, small inflammatory nodules at the site of inoculation. After the ninth day these nodules regressed and the animals remained well.

Rabbits. Three rabbits, when inoculated intradermally, developed no lesions. Four rabbits were inoculated intracerebrally with 0.3 cc. of the supernatant fluid of a 10 per cent emulsion of infected mouse brain. Of these 4 animals, 2 remained well. A third showed convulsions on the twelfth day, and at that time an opacity of the cornea had also developed. The fourth was found dead on the fifth day.

Five rabbits were inoculated on the scarified cornea with approximately 0.1 cc. of a 10 per cent emulsion of infected mouse brain. Only 1 of these animals remained normal. Each of the other 4 developed an opacity of the cornea which appeared 6 to 11 days after inoculation and gradually spread to involve the entire cornea. In one instance, extensive ulceration of both the cornea and the conjunctiva occurred. One of the rabbits was killed and the eye removed for study as soon as the opacity appeared. In the other 3 animals which developed a keratitis, partial paralysis of the posterior extremities occurred 2 to 3 days following the first appearance of the keratitis. In 2 of these animals this paralysis was unilateral, on the same side as the infected

cornea. In the third, both posterior extremities were involved. One of these paralyzed animals, in which the corneal opacity did not appear until the eleventh day and the paralysis not until the twelfth day, survived. The other 2 were killed when the paralysis was first observed, on the ninth and tenth days respectively.

Microscopic sections of 3 eyes, presenting well developed opacities of the cornea, showed a keratitis with an infiltration of polymorphonuclear leukocytes in the cornea and an extensive infiltration, composed of mononuclear cells and polymorphonuclear leukocytes, in the adjacent conjunctiva and the ciliary bodies. In the section of 1 eye the external half of the cornea and the adjacent conjunctiva was completely necrotic. Sections from the eye removed at the time of the first appearance of opacity showed only a slight inflammatory reaction, limited to the edge of the cornea and to the immediately adjacent conjunctiva. The epithelium covering the cornea of the latter eye was still intact, and at the edge of the cornea the epithelial cells were enlarged and undergoing proliferation. Several mitotic figures were seen. However, no intranuclear inclusions were observed.

In the brain of the rabbit which died on the fifth day after intracerebral inoculation a most intense inflammatory and necrotizing process was seen. The meninges showed an extensive exudate of lymphocytes, mononuclear cells, and polymorphonuclear leukocytes which was partially necrotic. In the outer part of the cortex of the cerebrum, at times continuous with the meninges, there were large areas of early necrosis, in and about which there was an infiltration of many polymorphonuclear leukocytes. In many nerve cells adjacent to these areas of necrosis, conspicuous large acidophilic inclusions were seen within the nuclei. These inclusions varied considerably in size, some completely filling the nucleus except for a deep-staining margin of chromatin. The staining of the inclusions with phloxine-methylene blue varied from light pink to lilac. In sections from the other 3 brains, 1 of a rabbit inoculated intracerebrally and 2 of animals inoculated on the scarified cornea, a well preserved meningeal exudate of lymphocytes and mononuclear cells was seen. In addition there were perivascular infiltrations of lymphocytes, focal accumulations of irregularly shaped mononuclear cells resembling those seen in the human brain, and occasional small, localized areas of

degenerating brain tissue, usually adjacent to the meninges. Nerve cells containing intranuclear acidophilic inclusions occurred, but these were far less numerous than in the brain of the rabbit dying on the fifth day.

Rats. Of 12 rats inoculated intracerebrally with 0.06 to 0.08 cc. of the supernatant fluid of a 10 per cent emulsion of infected mouse brain, 5 died 3 to 5 days following inoculation. The brains of 3 of these were examined microscopically. There occurred an infiltration of mononuclear cells in the meninges similar to that seen in the other species but with little necrosis of the exudate. Within the brain substance were seen changes comparable to those in the rabbit brains, but less severe. Intranuclear inclusions, though not numerous, were found in the nerve cells, always in areas where an inflammatory reaction was present.

Monkeys. Three rhesus monkeys, inoculated intracerebrally, remained well.

Chick Embryo. The virus was inoculated on the chorioallantoic membrane of the developing chick embryo. Large, discrete, opaque foci were observed when the membranes were examined on the second and third days following inoculation. Microscopically, the membranes showed a marked proliferation of the ectodermal layer of cells. Many of these cells contained intranuclear acidophilic bodies, and the lesions resembled, in general, those described for the virus of herpes simplex.

Neutralization of the Virus

Neutralization tests were carried out in mice with the R. T. virus and with a known herpes simplex virus (Rockefeller Institute H. F. strain). The serums used were an immune serum prepared by inoculating rabbits with the R. T. virus and two serums prepared by immunizing chickens (one with a known herpes virus (H. F. strain), and the other with a herpes virus modified by passage in the chicken embryo*). The technic used in the neutralization test was as follows: A 10 per cent suspension of virus was prepared by grinding infected mouse brains with a requisite amount of broth. After light centrifugation, the supernatant fluid was removed and serial tenfold dilutions in broth were made. Virus

* We are indebted to Miss Katherine Anderson, of the Department of Pathology, Vanderbilt University, for supplying us with the immune chicken serums.

dilutions of 10^{-2}, 10^{-3}, and 10^{-4} were used with undiluted immune serum. To 0.2 cc. of each virus dilution was added 0.4 cc. of serum. The mixtures were incubated for 2 hours at 37° C. and then injected intracerebrally in 0.03 cc. amounts into groups of 4 Swiss mice. The animals were watched daily during an observation period of 3 weeks for evidence of infection. Table I shows

TABLE I

Cross Neutralization Tests with Herpes Simplex Virus (Rockefeller Institute H. F. Strain) and with the R. T. Virus

Serum	Virus	Duration of life of test mice		
		Dilution of virus used in serum mixtures		
		10^{-2}	10^{-3}	10^{-4}
		days	*days*	*days*
Rabbit, immune to R. T. virus	R. T. virus	1, 12, S*, S	S, S, S, S	S, S, S, S
Rabbit, normal	R. T. virus	1, 4, 5, 5	5, 6, 6, 7	11, 13, 15, S
Rabbit, immune to R. T. virus	Herpes simplex	5, S, S, S	S, S, S, S	S, S, S, S
Rabbit, normal	Herpes simplex	4, 5, 5, 6	4, 6, 7, S	11, 19, S, S
Chicken, normal	R. T. virus	4, 4, 4, 6	7, 7, 10, 12	9, 11, S, S
Chicken, herpes (H. F.) immune	R. T. virus	5, 6, 6, 11	11, 12, S, S	13, S, S, S
Chicken, herpes (modified) immune	R. T. virus	6, 7, 10, S	S, S, S, S	S, S, S, S
Chicken, normal	Herpes simplex	3, 3, 3, 4	6, 7, 7, S	5, S, S, S
Chicken, herpes (H. F.) immune	Herpes simplex	6, 8, 9, S	S, S, S, S	5, S, S, S
Chicken, herpes (modified) immune	Herpes simplex	6, 7, 7, S	S, S, S, S	10, S, S, S

* S = Mouse remained well 21 days.

the results of the cross neutralization tests between a strain (H. F.) of known herpes simplex virus and the R. T. virus. It is readily apparent that immune serum to each virus was capable of neutralizing both viruses to the same extent and that the two viruses are immunologically identical.

DISCUSSION AND SUMMARY

A child, 4 weeks old, was brought to the hospital because of irritability, refusal to nurse, and twitchings of the left side of the body, and died on the fifth hospital day after a progressive accentuation of the cerebral symptoms. From the brain tissue taken at autopsy, a virus was isolated in mice which has been identified as that of herpes simplex.

During life the child's spinal fluid was sterile and no microörganisms were cultivated in dextrose infusion broth inoculated with an emulsion of the brain tissue. Furthermore, continued attempts to cultivate bacteria from the brains of mice, which died following the inoculation of infectious material originally derived from the human brain, have yielded negative results. The fact that we have been unable to show that the virus will pass through either Berkefeld N or V filters does not militate against its identification as herpes simplex, which is known to be filtrable only with difficulty.

The virus was highly infectious for mice following intracerebral inoculation, but only slightly so following subcutaneous inoculation. Rats, guinea pigs, and rabbits were also susceptible to infection with the virus. In these three species, an encephalitis resulted from intracerebral inoculation; rabbits, however, appeared more susceptible by this route than did guinea pigs and rats.

Following corneal inoculation in the guinea pig, an opacity developed which regressed. In the rabbit, however, the opacity may progress to ulceration of the cornea; and in three out of four animals showing corneal lesions a paralysis of the extremities developed. Intracutaneous injection of three guinea pigs was followed in each instance by the appearance of small nodules in the skin at the site of inoculation. No lesions developed when rabbits were injected intradermally.

Discrete, opaque foci appeared on the surface of the chorio-allantoic membrane of chicken embryos when they were inoculated with the virus.

Histological study of sections from the pons, medulla, and cerebellum of the brain of the child disclosed a meningo-encephalitis, the most conspicuous features of which were areas of necrosis, perivascular cellular infiltration, focal and diffuse inflammatory reaction, and nerve cell degeneration with intranuclear inclusions similar to those seen in known herpetic lesions.

Similar intranuclear inclusions were present in the brains of infected rabbits and rats. None was observed in the sections of the guinea pig brains studied, and few in those of mice. Inclusions were also present in large numbers in the proliferative lesions of the chick chorio-allantoic membrane. In all of the

experimental animals the cerebral lesions were essentially the same, although varying in intensity.

Cross neutralization was complete in tests performed with known herpes virus and antiserums. These results, together with those of the histological study and with the susceptibility of experimental animals, lead to the conclusion that the agent isolated is herpes simplex virus.

The work of Dodd, Johnston, and Buddingh,[16] and of Burnet and Williams,[17] has definitely shown the importance of aphthous stomatitis in children as a primary herpetic infection. It seems, therefore, that infants and children are highly susceptible to infection with herpes simplex virus, and one might anticipate that involvement of the central nervous system would be most likely to occur at this age. However, a review of the literature shows that this is the first report of a case of encephalitis from which herpes simplex virus was isolated in which the etiological significance of the virus has been established by the demonstration of typical herpetic inclusions in the human brain tissue.

REFERENCES

1. Epidemic Encephalitis: Etiology, Epidemiology, Treatment. Report of a Survey by the Mathewson Commission. Columbia University Press, 1929.

2. Gay, Frederick P., and Holden, Margaret. Isolation of a herpes virus from several cases of epidemic encephalitis. *Proc. Soc. Exper. Biol. & Med.*, 1933, **30**, 1051–1053.

3. Sabin, Albert B. Studies on the B virus. I: The immunological identity of a virus isolated from a human case of ascending myelitis associated with visceral necrosis. *Brit. J. Exper. Path.*, 1934, **15**, 248–268.

4. Levaditi, C., and Harvier, P. Étude expérimentale de l'encéphalite dite léthargique. *Ann. Inst. Pasteur*, 1920, **34**, 911–972.

5. Perdrau, J. R. The virus of encephalitis lethargica. *Brit. J. Exper. Path.*, 1925, **6**, 123–128.

6. Knauer, Hans, and Jaensch, P. A. Nachweis einer einheitlichen Ätiologie bei den verschiedenen Formen der Encephalitis im Anschluss an Infektionskrankheiten im Kindesalter. *Klin. Wchnschr.*, 1930, **9**, 2049–2051.

7. Radici, M. Ricerche sul virus encefalitico. *Riv. di clin. pediat.*, 1931, **29**, 944.

8. Hissard, M. René. Inoculation positive, à la cornée du lapin, de liquide

céphalorachidien d'une malade atteinte de méningite aiguë, bénigne à "lymphocytose surabondante." *Bull. Soc. franç. de dermat. et syph.*, 1933, **40**, 1319–1323.

9. Rupilius, Karl, and Szekely, Josef. Zum Nachweis des Enzephalitisvirus durch Hornhautimpfung. *Jahrb. f. Kinderh.*, 1934, **142**, 351–358.

10. Doerr, Robert, and Hallauer, Curt. Handbuch der Virusforschung. Julius Springer, Vienna, 1938, **1**, 42.

11. Zurukzoglu, St. Ueber das Vorkommen von Herpesvirus im Liquor cerebrospinalis. *Zentralb. f. Bakteriol.*, 1937, **139**, 86–90.

12. Hoff, H., and Pötzl, O. Über Schienen-Enzephalitis. *Wien. med. Wchnschr.*, 1937, **87**, 374–376.

13. Kreis, Boris. Présence du virus herpétique dans la salive de parkinsoniens post-encéphalitiques. *Compt. rend. Soc. de biol.*, 1938, **127**, 108–109.

14. Levaditi, C., Harvier, J., and Nicolau, S. Étude expérimentale de l'encéphalite dite "léthargique." *Ann. Inst. Pasteur*, 1922, **36**, 63–104.

15. Dawson, James R., Jr. Cellular inclusions in cerebral lesions of lethargic encephalitis. *Am. J. Path.*, 1933, **9**, 7–16.

16. Dodd, Katharine, Johnston, Leland M., and Buddingh, G. John. Herpetic stomatitis. *J. Pediat.*, 1938, **12**, 95–102.

17. Burnet, F. M., and Williams, Stanley W. Herpes simplex: A new point of view. *M. J. Australia*, 1939, **1**, 637–642.

DESCRIPTION OF PLATE

Plate 13

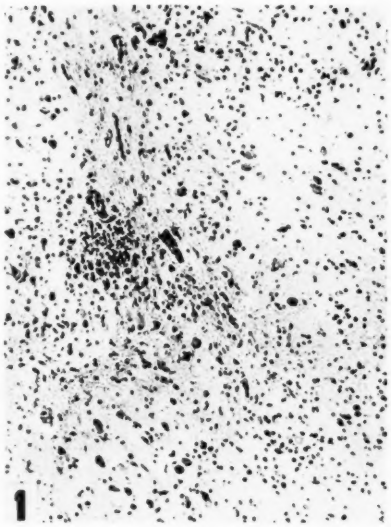
Fig. 1. Focus of proliferated glia in pons. This and all other figures were made from sections of human brain. × 100.

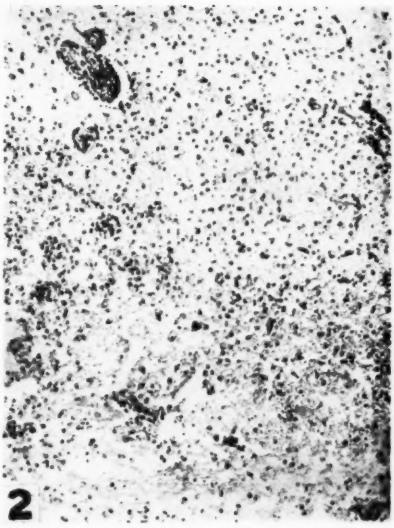
Fig. 2. Area of necrosis and inflammation in pons. Perivascular infiltration of lymphocytes. × 100.

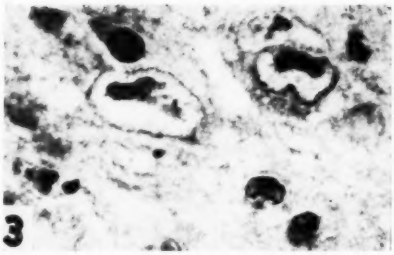
Figs. 3 and 4. Nerve cells in pons showing large intranuclear inclusions separated from nuclear membranes by clear zones. × 720.

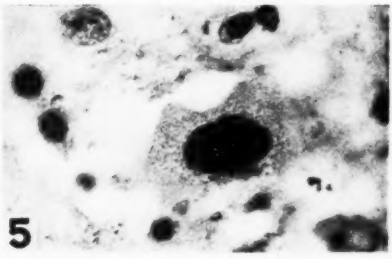
Fig. 5. Nerve cell in pons showing large granular inclusion filling the nucleus. × 720.

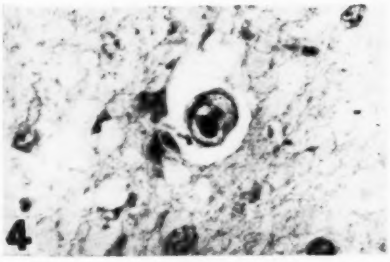
Fig. 6. Nerve cell in cerebellum showing a large granular inclusion filling the nucleus. Dots of nuclear chromatin on the nuclear membrane. × 720.

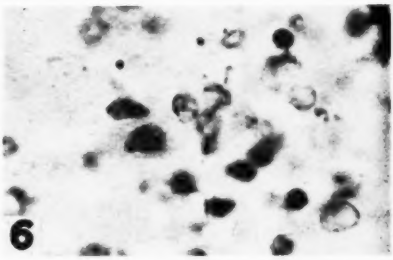












Smith, Lennette and Reames

Herpes Simplex and Acute Encephalitis

THE PRODUCTION OF NEURONAL INJURY AND NECROSIS WITH THE VIRUS OF POLIOMYELITIS IN RABBITS DURING INSULIN HYPOGLYCEMIA*

BENJAMIN P. SANDLER, M.D.
(From the Morrisania City Hospital, New York, N.Y.)

While engaged in a clinical study of chronic hypoglycemia in patients of all ages I was impressed with the fact that several patients gave histories of attacks of poliomyelitis with residual paralysis. It occurred to me that a disturbance in carbohydrate metabolism could be a factor in susceptibility to infection with the virus of poliomyelitis, especially since, during hypoglycemia, cellular oxidation will be reduced. It has been demonstrated both in men [1] and dogs [2] that the oxygen uptake of the central nervous system falls during insulin hypoglycemia. Using the Barcroft-Warburg technic, various workers [3,4,5] have shown that excised pieces of brain, peripheral nerve and meninges utilize less oxygen as the amount of glucose in the nutrient medium is reduced. Wortis [5] also found that, weight for weight, the nervous tissues of the young in any species consume more oxygen than those of the adult and he concluded that the young are more vulnerable to hypoglycemia than the adult.

The rhesus monkey is very susceptible and the rabbit resistant to the virus of poliomyelitis. Without knowing the blood sugar range in these animals it was suspected that the blood sugar in the monkey reached lower levels than in the rabbit. These suspicions were found to have a basis in fact through the investigations of Jungeblut and Resnick,[6] who studied glucose tolerance in monkeys, and du Vigneaud and Karr [7] who studied glucose tolerance in rabbits. In the monkey, values were obtained as low as 50 mg. per 100 cc., whereas, in the rabbit, the blood sugar was never observed to fall below 100 mg. per 100 cc. Cori [8] has stated that the blood sugar of mammals normally remains in the neighborhood of 100 mg. In twenty-five determinations made on fasting rabbits I have never observed the blood sugar to be below 100 mg. It was therefore concluded that the resistance of the

* Received for publication April 15, 1940.

rabbit might be associated with the fact that its blood sugar never fell below 100 mg. and that at this concentration cellular oxidation in the nervous system and in other organs would be maintained at such a level as to enable the cells to protect themselves against invasion by the virus. It was thought that if hypoglycemia were induced in the rabbit and the virus of poliomyelitis then injected, evidence of action by the virus might be obtained.

Methods

Rabbits were fasted for 24 hours. A fasting blood sugar specimen was drawn and 0.6 to 0.8 unit protamine insulin per Kg. of body weight was injected subcutaneously. Such a dose usually depressed the blood sugar to around 45 mg. per 100 cc. in 1 to 3 hours and maintained hypoglycemia from 3 to 5 hours, and sometimes longer. Blood sugar specimens were drawn at various intervals from an ear vein and sugar estimated after the macromethod of Folin and Wu. If symptoms of severe hypoglycemia, such as muscular weakness, exophthalmos, restlessness, hyperexcitability and convulsions were noted, a small amount of 20 per cent glucose solution was injected intravenously or intraperitoneally. About an hour after the insulin injection, 0.4 cc. of a 30 per cent suspension of monkey cord virus was injected intracerebrally under novocain anesthesia. Rectal temperatures were taken at frequent intervals. During hypoglycemia the temperature may fall from 1 to 4 degrees, due, no doubt, to reduced bodily oxidation of glucose. Food was withheld after inoculation. Some rabbits received injections of insulin on successive days. Intracerebral injections of virus were not repeated. Rabbits receiving intranasal instillations of virus were prepared in a similar manner: three doses of 1 cc. of a 50 per cent suspension were instilled into each nostril every 2 hours on the first day and one dose daily for 1 to 3 days thereafter. Insulin was injected whenever nasal instillations were made on successive days. The virus used in these experiments was the MV strain of the Rockefeller Institute.

Results

*I. Rabbits Injected with Monkey Cord Virus Suspensions.**

A. Intracerebral Injection. Four rabbits inoculated by

* In preparing virus suspensions from infected rabbits only the cord was used.

this route died in 14 hours, 2 days, 6 days, and 15 days after inoculation. Severe lesions were found in all.

REPRESENTATIVE PROTOCOLS

Rabbit No. 1.

11/16/38. 11:00 a.m.: blood sugar, 102 mg. per 100 cc.; t. 103°; insulin, 2 units.
1:30 p.m.: blood sugar, 38 mg.; 0.4 cc. virus suspension injected.
3:30 p.m.: convulsions; glucose administered.
4:00 p.m.: rabbit fully recovered.
9:00 p.m.: blood sugar 226 mg.; t. 104.5°; all extremities were weak; eyelids drooped; respirations rapid.

11/17/38. 2:30 p.m.: t. 103.4°; refused food; all extremities paretic; condition worse.

11/18/38. 10:00 a.m.: t. 102.4°; extremities flaccid.
2:30 p.m.: died.

Autopsy: Cut surface of medulla and cord at various levels showed gray matter to be hemorrhagic and soft. Microscopic examination at various levels revealed severe necrosis of the anterior horn cells and in some sections the neurons had disappeared (Fig. 1).

Rabbit No. 13.

3/11/39. 9:00 a.m.: blood sugar, 120 mg. per 100 cc.; t. 103°.
9:15 a.m.: insulin, 1 unit.
10:10 a.m.: 0.4 cc. virus suspension injected.
10:15 a.m.: t. 102°; blood sugar, 100 mg.
11:45 a.m.: blood sugar, 66 mg.
12:30 p.m.: t. 102.8°.
1:30 p.m.: blood sugar, 40 mg.; convulsions; glucose administered.
2:30 p.m.: t. 100°.
4:00 p.m.: looked ill; limp; eyelids drooped; coarse tremors of hind legs.
11:00 p.m.: died.

Autopsy: Cord grossly hyperemic and edematous. Microscopic examination showed widespread moderate to severe necrosis of anterior horn cells (Fig. 2).

B. INTRANASAL INSTILLATION. Three rabbits were inoculated by this route. One died on the sixth day and the other 2 were killed on the sixth and twelfth days. Lesions were found in all, but were most severe in the rabbit that died spontaneously.

REPRESENTATIVE PROTOCOL

Rabbit No. 18.

2/18/39. 5:45 p.m.: t. 102.7°; blood sugar, 105 mg. per 100 cc.; insulin, 0.8 unit.

6:40 p.m.: t. 100.6°; blood sugar, 60 mg.
7:00 p.m.: 1 cc. virus suspension instilled into each nostril.
8:05 p.m.: t. 100.5°.
8:30 p.m.: blood sugar, 80 mg.
8:50 p.m.: 1 cc. virus suspension instilled into each nostril.
10:10 p.m.: t. 101.6°.
11:15 p.m.: 1 cc. virus suspension instilled into each nostril.

2/19/39. 12:20 a.m. t. 102.2°.

2/20/39. t. 102.0°.

2/21/39. t. 102.3°.

2/22/39. t. 102.0°.

Autopsy: Surface of brain and cord hemorrhagic and edematous; cut surface of cord, especially in the thoracic region, showed gray matter to be prominent and hyperemic. Microscopic examination revealed scattered neuronal necrosis most marked in the thoracic levels (Fig. 3).

II. Rabbits Injected with Suspensions of Rabbit Cord Virus.

A. INTRACEREBRAL INJECTION. Two rabbits inoculated by this route died spontaneously on the third and fourth days. Both showed severe lesions.

REPRESENTATIVE PROTOCOL

Rabbit No. 16.

3/27/39. 7:40 p.m.: t. 103°; blood sugar, 110 mg. per 100 cc.; insulin, 1.6 unit.
8:30 p.m.: 0.4 cc. cord suspension from rabbit No. 13.
9:15 p.m.: t. 104.2°.
10:25 p.m.: hypoglycemic symptoms; blood sugar, 45 mg.; glucose administered.

3/28/39. 2:00 p.m.: t. 102°; looked ill; eyelids drooped; no paralysis.

3/29/39. 2:00 p.m.: t. 101°; condition worse; paresis of extensor muscles of head and neck.

3/30/39. Died.

Autopsy: Cut surface of cord showed gray matter to be prominent and hemorrhagic. Microscopic examination revealed severe neuronal necrosis at various levels.

B. INTRANASAL INSTILLATION. Two rabbits were inoculated by this route. One died on the third day and the other was killed on the fifth day. Both showed lesions.

REPRESENTATIVE PROTOCOL

Rabbit No. 17.

4/1/39. 7:00 p.m.: t. 102.2°; blood sugar, 100 mg. per 100 cc.; insulin, 1.5 unit.

7:30 p.m.: 1 cc. cord suspension from rabbit No. 16 instilled into each nostril.
8:10 p.m.: t. 102°.
8:40 p.m.: blood sugar, 60 mg.
9:00 p.m.: 1 cc. cord suspension instilled into each nostril.
10:00 p.m.: t. 100.9°.
10:55 p.m.: t. 101.5°.
11:45 p.m.: t. 101.6°; blood sugar, 97 mg.

4/2/39. 3:20 p.m.: t. 104°; blood sugar, 120 mg.; insulin, 1.7 unit.
5:00 p.m.: 1 cc. cord suspension instilled into each nostril.
5:10 p.m.: blood sugar, 55 mg.
5:40 p.m.: t. 101.2°.
6:30 p.m.: t. 99.6°.
6:40 p.m.: blood sugar, 20 mg.
6:45 p.m.: convulsions; glucose administered.
6:55 p.m.: rabbit fully recovered.
7:20 p.m.: 1 cc. cord suspension instilled into each nostril.
7:45 p.m.: t. 98.4°.
9:10 p.m.: t. 100.4°.

4/3/39. t. 104.0°; no paralysis.

4/4/39. Died.

Autopsy: Cord hemorrhagic and soft. Microscopic examination showed scattered severe neuronal necrosis at various levels (Fig. 4).

III. Controls.

A. Insulin Alone. Several rabbits dying in insulin shock showed no neuronal changes.

B. Monkey Virus Suspensions Alone. No lesions were observed after intracerebral injection.

C. Innocuous Monkey Cord Suspensions and Insulin. Sixteen rabbits were injected intracerebrally in this group. There were no spontaneous deaths and no lesions were found on microscopic examination.

D. Normal Rabbit Cord Suspension and Insulin. No deaths and no lesions were found on microscopic examination.

E. Inoculation of Monkeys with Suspensions of Rabbit Cord Virus.

Representative Protocols

Monkey No. 3.

MV virus
↓ (intracerebrally)
Rabbit No. 13
↓ (intracerebrally)
Monkey No. 3

3/12/39. t. 102.5°; injected with cord suspension from rabbit No. 13.

3/13/39. t. 102.0°.

3/14/39. t. 102.0°.

3/15/39. t. 103.5°.

3/16/39. t. 102.0°.

3/17/39. t. 103.5°.

3/18/39. t. 102.0°.

3/19/39. t. 102.5°.

3/20/39. t. 105.7°; tired easily; respirations rapid.

3/21/39. t. 104.4°; tremors; excited.

3/22/39. t. 100.0°; hair ruffled; marked tremors; paralysis of left lower extremity.

3/23/39. t. 100.5°; paralysis of both lower extremities.

3/24/39. t. 100.5°; paralysis of all extremities; killed.

Autopsy: Cord hemorrhagic and markedly edematous. Microscopic examination showed interstitial and perivascular infiltrations, and severe necrosis of anterior horn cells (Fig. 5).

Monkey No. 4.

MV virus
↓ (intracerebrally)
Rabbit No. 13
↓ (intracerebrally)
Rabbit No. 16
↓ (intranasally)
Rabbit No. 19
↓ (intranasally)
Monkey No. 4

4/24/39. t. 102.8°; inoculated intranasally with cord suspension from rabbit No. 19.

4/25/39. t. 101.8°.

4/26/39. t. 102.0°; hair ruffled; less active; left upper extremity appeared limp.

4/27/39. t. 100.6°; cry was weak; left upper extremity improved.

4/28/39. t. 100.6°.

4/29/39. t. 99.4°.

4/30/39. t. 101.0°.

5/1/39. t. 102.8°.

5/2/39. t. 102.6°; left upper extremity not used as often as the right, and was weaker than the right.

5/3/39. Killed.

Autopsy: All cut surfaces of cord showed pin point hemorrhages, more marked on left side of cord in the cervical region. Microscopic

examination showed perivascular edema and swelling of vessel walls; focal collections of polymorphonuclears and round cells; cells in the central canal; scattered neuronal necrosis which was most marked in the left anterior horn of the cervical region (Fig. 6).

The severe lesions following nasal instillation of suspensions of virus in hypoglycemic rabbits obviate any possibility that the neuronal changes resulted from intracerebral injections of the material used in the preparation of suspensions. That no spontaneous deaths occurred in the controls injected with insulin and innocuous cord suspensions, and that eight of eleven rabbits injected with insulin and virus suspensions died, indicate that the rabbits in the latter group were infected.

It can be stated with certainty that one to three injections of insulin, using doses of one to two units, will not injure neurons in any way that can be demonstrated histologically, even if the blood sugar is depressed to convulsive level. Rabbits dying in hypoglycemic shock showed normal neurons throughout. Weil, Liebert and Heilbrunn [9] injected as much as 60 units of insulin into rabbits in one day and were unable to produce demonstrable changes in the neurons. For example, they produced forty-five seizures in one rabbit using a total of 59 units of insulin and found no histopathological changes in the neurons. They found some changes after 70 to 150 units given in divided doses, and severe changes after 200 to 400 units. The significant factor in the production of changes is the cumulative effect of repeated injeotions of large doses of insulin.

Lesions in the Rabbit. Severe lesions in the medulla and cord may be noted in rabbits dying as early as 13 hours after intracerebral inoculation. The widespread distribution of lesions in such rabbits demonstrates the ability of the virus to invade in a very short time. Apparently the virus encounters little or no resistance during the period of hypoglycemia. Lesions are distributed unevenly much as they occur in humans and monkeys. One side of the cord may show more marked involvement than the other. Lesions may be present in the cervical and lumbar regions and absent in the thoracic. The presence of neuronal injury is the striking feature of the pathological picture. Capillary, arterial, and venous engorgement is prominent, accounting

for the hyperemic appearance of the cord on gross examination. Neuronophagia was not observed. Perivascular and interstitial infiltrations were absent. In mild injury the neurons appear swollen, the Nissl bodies are less sharply defined, and the cell as a whole takes a lighter and more eosinophilic stain. In moderate injury the neurons are pale and swollen and present a "washed-out" appearance with absence of Nissl bodies; the nuclei are clearer, granular, and may contain inclusion bodies. In severe injury the neurons are shrunken, with pyknotic and fragmented nuclei; the cytoplasm is undergoing dissolution and in some instances is barely distinguishable from the ground substance. Often a mass of debris represents the site of the neuron and sometimes it may completely disappear.

The presence, exclusively, of neuronal injury and the absence of perivascular and interstitial infiltrations represent the fundamental nature of infection with a neurotropic virus. Hurst [10] has emphasized the fact that the injury to the neuron is the essential lesion in a neurotropic virus disease such as poliomyelitis. The following is quoted from his article: "In all probability viruses are obligatory intracellular parasites. If, therefore, the adjective neurotropic as applied to certain viruses has any significance, we should logically expect such viruses to be capable of a primary and direct attack on the nerve cells. Such is in fact the case. Yet the older literature on so typical a neurotropic virus disease as poliomyelitis ascribed the lesion in nerve cells wholly or in large part to impaired nutrition, resulting from interstitial inflammation . . . The microscopical appearances of nerve cells affected by a virus vary with the acuteness or otherwise of the disease process. In hyperacute and acute conditions, and hence commonly in experimental infections . . . the type lesion is acute necrosis of the neurons. The cytoplasm is shrunken and strongly eosinophilic, the nucleus pyknotic, karyorrhectic, fading or absent; in the brief pre-necrotic phase various types of neuronal change described by Nissl may be seen, but especially the so-called ischemic cell-change and the 'severe cell-change'."

Course of the Disease in Rabbits. The animal may show evidence of infection in 8 to 10 hours after intracerebral inoculation. After nasal inoculation signs of infection may not be evident until

24 to 48 hours have elapsed. Symptoms due to infection need not be confused with hypoglycemic symptoms since the latter appear in 1 to 5 hours after the injection of insulin. When infection occurs, the rabbit becomes less active, shows no desire for food, the eyelids droop, and in general the animal looks ill. Respirations may be increased. Fibrillary twitchings or coarse tremors may be present. Tremors may be more evident when the rabbit is grasped and held in the air. Some rabbits give a sensation of limpness and do not struggle when lifted off the table. The temperature may rise to 104° F. after 24 to 48 hours and return to normal after 72 hours. Paralysis was noted twice among eleven rabbits. One rabbit had weakness of all extremities after 24 hours with complete paralysis after 44 hours. A second rabbit, which was injected intracerebrally with a virus suspension from an infected rabbit, developed paresis of the extensor muscles of the head and neck on the second day and died on the third day. In some rabbits frank signs of infection are lacking although lesions will be found on histological examination. Close inspection may reveal some awkwardness in gait or unsteadiness after a few days.

Discussion

The disease in the rabbit differs, obviously, in many respects from the disease in the monkey. The incubation period and clinical course are shorter. That these differences exist need not be at all surprising if the significance of hypoglycemia is recognized, since the disturbance in carbohydrate metabolism as it exists in the monkey was not reproduced at all in the rabbit. In the monkey, according to my concept of the factor of susceptibility, the virus grows and invades only when the blood sugar falls low enough to permit growth and invasion. Such periods of hypoglycemia may occur several times during 24 hours. Resistance to infection and variability in incubation period in the monkey may thus be due, in part, to variations in the degree of the disturbance in carbohydrate metabolism.

In these experiments the virus is enabled to grow and invade only during the period of induced hypoglycemia. As the effect of the insulin wears off and the blood sugar rises to normal levels, the virus is apparently prevented from growing and invading with

the rapidity reached during hypoglycemia. Those neurons harboring an overwhelming dose of virus will be unable to destroy it and will eventually die because intracellular metabolic processes will be disrupted. Death of the animal results from destruction of neurons in vital medullary centers. Death occurring as soon as 14 hours after inoculation may be attributed to severe and prolonged hypoglycemia, to a potent virus, and to marked involvement of medullary centers. If the disturbance in carbohydrate metabolism as it exists in the monkey were reproduced in the rabbit, the lesions and clinical course would probably be duplicated.

Summary

1. It is suggested that disturbance in carbohydrate metabolism, especially hypoglycemia, may be an important factor in determining susceptibility to the virus of poliomyelitis, both in man and in the monkey. Hypoglycemia reduces cellular oxidations, causing a cellular asphyxia of mild, moderate, or severe degree depending on the degree of hypoglycemia. That this asphyxia lowers the resistance of the individual cell and of the organism in general to invasion by the virus may be the mechanism of increased susceptibility.

2. The blood sugar of the rhesus monkey during tolerance tests has been observed to fall as low as 60 mg. per 100 cc. It is suggested that this hypoglycemia is responsible for the susceptibility of the rhesus monkey to the virus of poliomyelitis.

3. The blood sugar of the fasting rabbit is maintained at or above 100 mg. per 100 cc. By depressing the blood sugar to 60 mg. or less, it has been possible to produce neuronal injury in the anterior horns with the virus of poliomyelitis, both by intracerebral and by intranasal inoculation. Suspensions prepared from the spinal cords of infected rabbits, when injected into monkeys by the intracerebral route, cause primary neuronal injury and necrosis along with inflammatory changes.

REFERENCES

1. Dameshek, William; Myerson, Abraham, and Stephenson, Caroline. Insulin hypoglycemia; mechanism of the neurologic symptoms. *Arch. Neurol. & Psychiat.*, 1935, **33**, 1–18.

2. Himwich, H. E., and Fazekas, J. F. The effect of hypoglycemia on the metabolism of the brain. *Endocrinology*, 1937, **21**, 800–807.

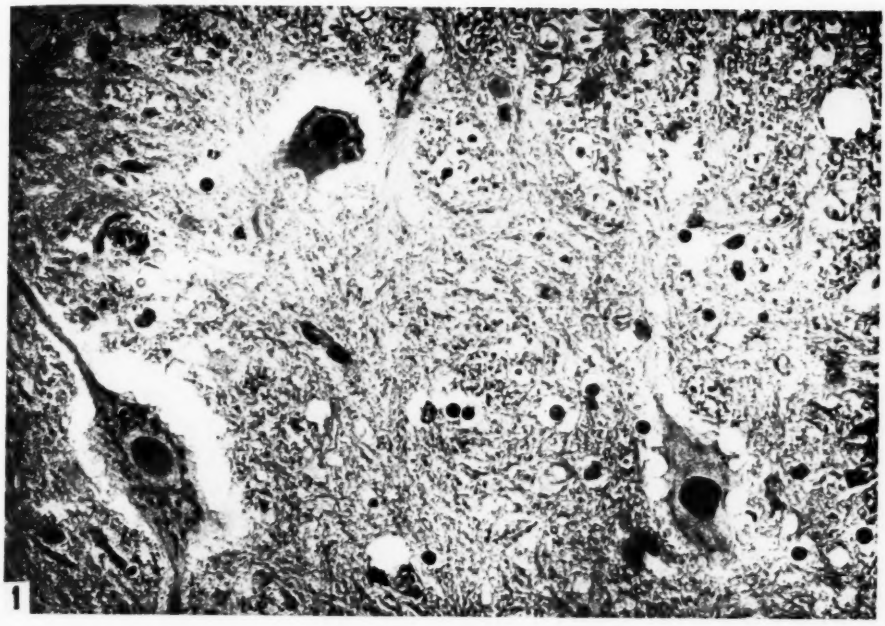
3. Holmes, E. G. Oxidations in central and peripheral nervous tissue. *Biochem. J.*, 1930, **24**, 914–925.
4. Dickens, Frank, and Greville, Guy Drummond. Metabolism of normal and tumour tissue. VIII. Respiration in fructose and in sugar-free media. *Biochem. J.*, 1933, **27**, 832–841.
5. Wortis, S. Bernard. Respiratory metabolism of excised brain tissue. I. The respiratory quotient; carbohydrate and lactic acid utilization. *Am. J. Psychiat.*, 1934, **13** n.s., 725–732.
6. Jungeblut, Claus W., and Resnick, Rose. Blood sugar levels and dextrose tolerance in experimental poliomyelitis. *Am. J. Dis. Child.*, 1936, **51**, 91–98.
7. du Vigneaud, Vincent, and Karr, Walter G. Carbohydrate utilization. I. Rate of disappearance of d-glucose from the blood. *J. Biol. Chem.*, 1925, **66**, 281–300.
8. Cori, Carl F. Mammalian carbohydrate metabolism. *Physiol. Rev.*, 1931, **11**, 143–275.
9. Weil, A., Liebert, E., and Heilbrunn, G. Histopathologic changes in the brain in experimental hyperinsulinism. *Arch. Neurol. & Psychiat.*, 1938, **39**, 467–481.
10. Hurst, E. Weston. The newer knowledge of virus diseases of the nervous system: a review and an interpretation. *Brain*, 1936, **59**, 1–34.

DESCRIPTION OF PLATES

PLATE 14

FIG. 1. Rabbit No. 1. First passage, intracerebral inoculation. Thoracic segment; anterior horn cells in varying degrees of necrosis. Note loss of Nissl bodies. The cytoplasm is undergoing dissolution and the cell borders have become frayed. Capillaries are dilated. All sections were stained with hematoxylin and eosin. × 390.

FIG. 2. Rabbit No. 13. First passage, intracerebral inoculation. Thoracic segment; anterior horn cells in varying degree of necrosis. Nissl bodies stain poorly and are in process of dissolution. Note swelling of axons. Capillaries are dilated. × 370.



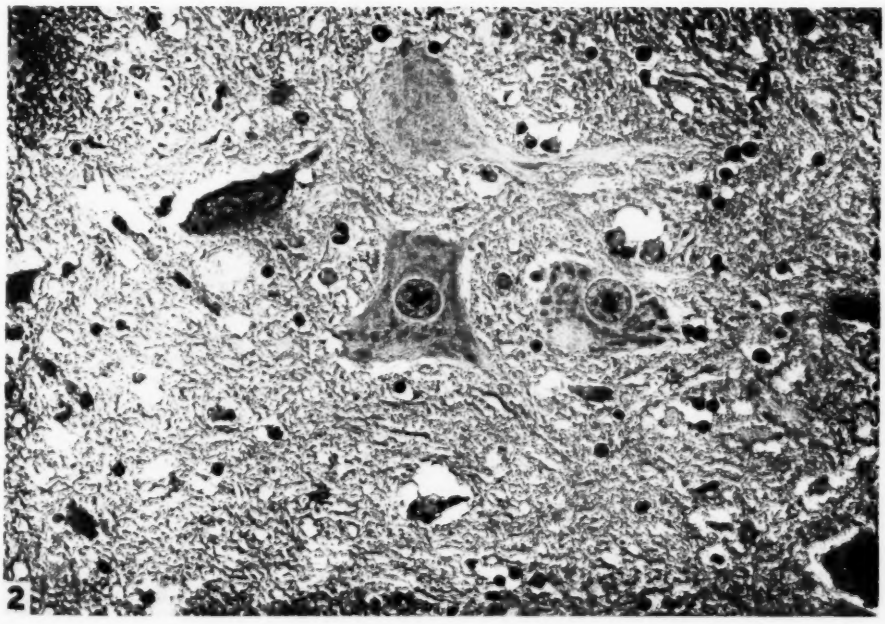
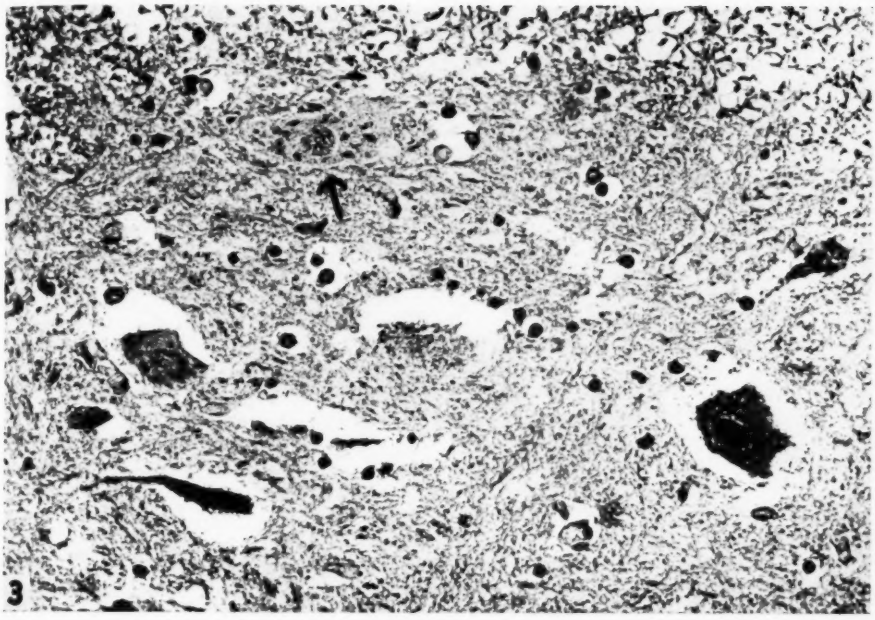


PLATE 15

FIG. 3. Rabbit No. 18. First passage, intranasal inoculation. Thoracic segment; moderate to severe necrosis of anterior horn cells. One neuron (marked by an arrow) is barely distinguishable from the ground substance. × 370.

FIG. 4. Rabbit No. 17. Third passage, intranasal inoculation. Motor neurons from medulla. Primary neuronal injury with loss of Nissl bodies. The pale areas take an eosinophilic stain with hematoxylin and eosin. × 360.


3

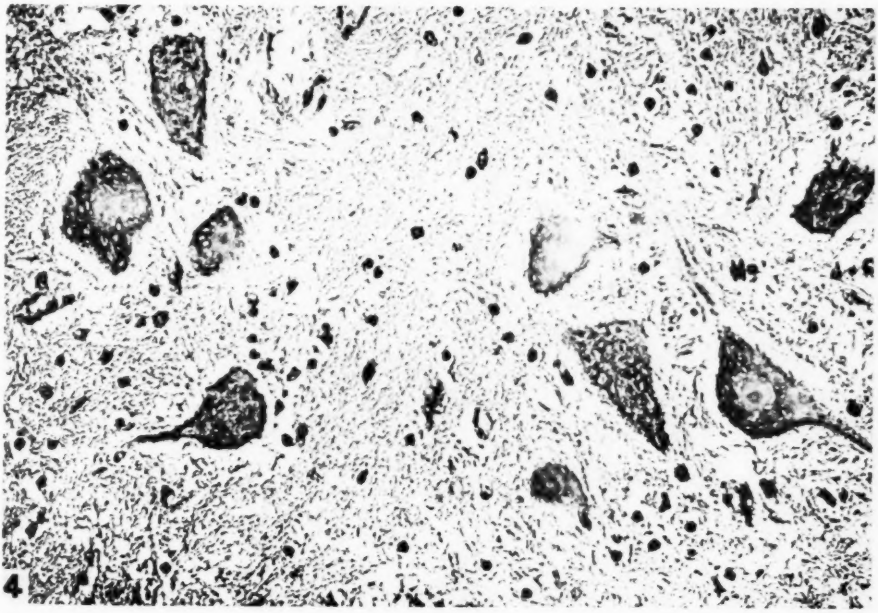
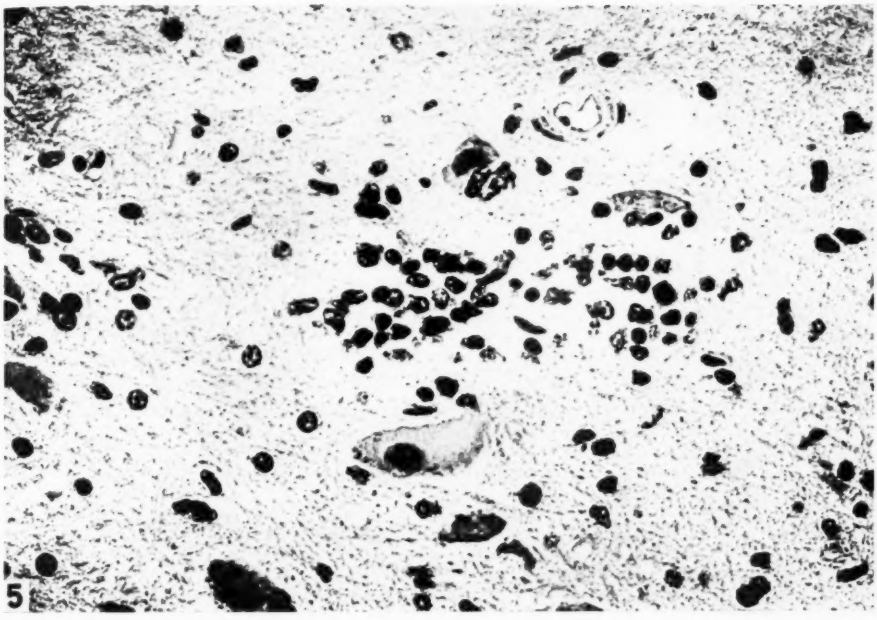
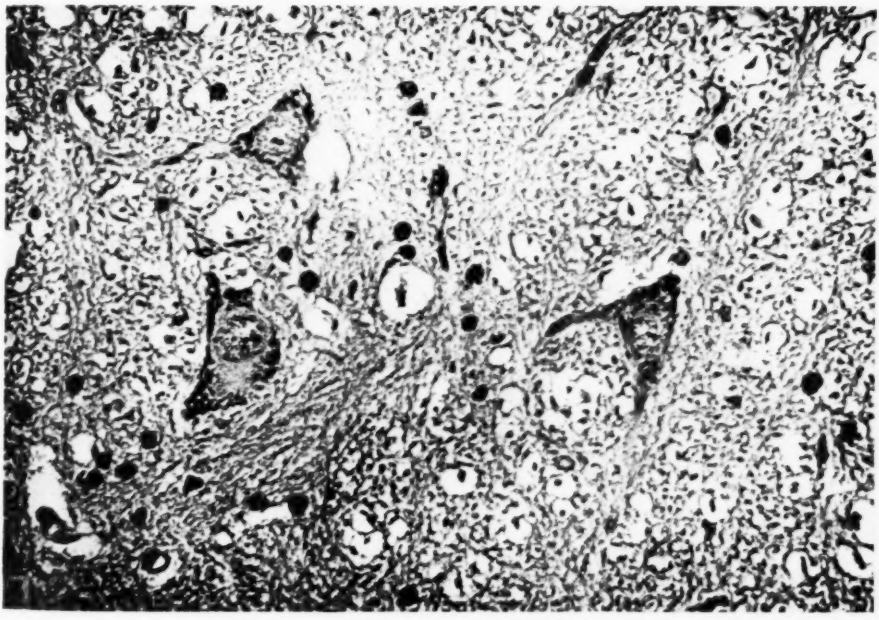

4

PLATE 16

FIG. 5. Monkey No. 3. Thoracic segment; area of infiltration with polymorphonuclear leukocytes and small round cells. Severe neuronal necrosis and neuronophagia. × 650.

FIG. 6. Monkey No. 4. Cervical segment; motor neurons from left anterior horn. Severe primary neuronal necrosis without interstitial infiltration. × 390.





MUSCULAR DYSTROPHY IN BILIARY FISTULA DOGS; POSSIBLE RELATIONSHIP TO VITAMIN E DEFICIENCY*

K. M. BRINKHOUS, M.D., AND E. D. WARNER, M.D.

(From the Department of Pathology, State University of Iowa, Iowa City, Iowa)

Many of the chronic biliary fistula dogs which we have studied in our vitamin K experiments [1] developed marked muscular weakness, followed by atrophy of skeletal muscles. Examination of the affected muscles [2] revealed lesions similar to those of the nutritional muscular dystrophy which has been shown recently to be related to a vitamin E deficiency. Although our chronic biliary fistula dogs were maintained on an adequate diet, faulty absorption, incident to the absence of bile in the intestine, resulted in a deficiency of at least two of the fat-soluble vitamins; vitamin K, as indicated by a hemorrhagic tendency, and vitamin D, as indicated by the gradual development of osteomalacia. In view of the recent work on vitamin E and nutritional muscular dystrophy, it appears probable that an analogous deficiency of vitamin E was responsible for the muscular dystrophy in these animals.

MATERIALS AND RESULTS

The gallbladder-renal type of fistula was used.[3] The animals were fed a diet consisting either of hospital scraps, of dog chow (Purina), or a standard mixed diet previously described.[1] Unoperated control dogs maintained on these same diets remained entirely normal and developed no muscle lesions. In the untreated biliary fistula animals, osteoporosis, duodenal ulcers, and intestinal disturbances, as described by Hawkins and Whipple,[4] as well as the hemorrhagic diathesis due to hypoprothrombinemia,[5] were frequent complications. In addition, it was noted that unless bile was fed by mouth, the animals regularly developed muscle lesions within 6 to 8 months after the fistula was established.

* Aided by a grant from the John and Mary R. Markle Foundation. Funds for technical assistance were supplied by the Graduate College, State University of Iowa.

Received for publication April 24, 1940.

TABLE I
Muscular Dystrophy in Biliary Fistula Dogs

Dog No.	Sex	Duration of biliary fistula	Muscle weakness	Muscle lesions	
				Necrotic lesions	Simple atrophy
		months			
1	F	6	None	+	o
2	F	7½	Slight	+++	Slight
3	F	7½	Slight	+	+
4	F	9½	Marked, unable to walk last 12 weeks	++	+++
5	M	11	Marked, unable to walk last week	++	+
6	F	11½	Marked, unable to walk last 2 weeks	+	+
7	F	12	Marked, unable to walk last 8 weeks	+	++
8	M	13	Slight	++	+
9	M	13½	Slight	++	+
10	M	14	Marked, unable to walk last 4 weeks	++	++
11	M	14½	Marked, unable to walk last week	++	++
12	F	26½	Slight	+	+
13	F	32	Marked, unable to walk last 2 weeks	++	+

Table I lists the animals in which a careful study of the dystrophic muscle changes was made. Ten of these animals received no bile or bile salts at any time during their course and the remaining three (dogs 2, 12, and 13) received none within the 6 months prior to study of the muscles. The dystrophy varied markedly in severity in this group. In dog 4, paresis was so extreme 6½ months after operation that the animal was able to walk only with difficulty. On the other hand, dog 12 was observed for over 2 years, during which time only moderate muscular weakness developed. This was insufficient to impair seriously the animal's activity. In dog 1 there were no overt symptoms in 6 months. However, in this animal as well as in all the others of this group, well developed muscular lesions were observed on microscopic examination.

The primary lesion of the skeletal muscles appeared to be a focal hyaline necrosis of a whole fiber or more commonly a short segment of it (Fig. 1). Necrosis was followed by phagocytosis and absorption of the necrotic sarcoplasm, and eventual collapse of the fibers. Replacement fibrosis was noted in advanced cases. In addition to the necrosis, there was variable and often marked proliferation of the subsarcolemmic nuclei, both about the sites of necrosis and in otherwise unaffected fibers. The nuclei were oval or elongated, at times centrally placed in the fiber, and occasionally in chains of six to ten. Little active regeneration of the destroyed portions of the fibers was observed, however.

Simple atrophy of many of the muscle fibers unaffected by necrosis was present in some of the muscle groups, but this was a much less consistent finding than were the necrotizing lesions and the nuclear proliferation. In a very few instances in the markedly atrophic muscles, there was also a slight degree of adipose tissue replacement. Aside from the shrinkage in size and some pallor, the affected muscles were not abnormal to gross examination.

These degenerative, proliferative and atrophic changes were distributed in patchy fashion throughout the involved muscles. In this group of animals, no muscle groups were exempt, but those of the posterior extremities were affected most severely. This corresponded with the muscles which showed the most marked paresis during life. The patchy distribution of the lesions may account for the lack of any very exact correlation between the degree of paresis and the extent of the lesions in the sections studied. As a rule, only one section of each muscle group was taken. More extensive sampling might have resulted in a more exact correlation.

With the appearance of the atrophic muscle changes approximately 7 to 9 months after operation, many of the animals began to lose weight. In some this was 20 per cent or more of the original preoperative weight, although food consumption was adequate. The extent of the weight loss tended to parallel, in general, the degree of muscular atrophy.

The nervous system (brain, spinal cord and peripheral nerves) of seven of the animals of this group was studied carefully. Sections were stained both with hematoxylin and eosin and with stains for myelin sheaths. In addition stains for nerve endings (Ranvier) were studied in several of the dogs. No evidence of neuronal or glial changes, or of degeneration of tracts, peripheral nerves or motor end-plates could be found.

No lesions of cardiac muscle or of smooth muscle of the uterus, alimentary tract, bronchi, blood vessels or bile ducts were observed.

The testes of the males of this group of animals showed marked degenerative changes of the germinal epithelium, with impairment of or complete loss of spermatogenesis. In three of the animals these changes were extreme. Many of the seminiferous tubules

were small and contained few or no germ cells. In the testes of dog 8 these changes were less extreme, and all stages of degeneration of the germinal epithelium, including extensive multinucleated giant cell formation (Fig. 2), similar to that described by Mason [6] in vitamin E deficiency in rats, were noted.

One animal, not listed in Table I, is of particular interest because of the effects of bile feeding. About the twelfth month after the establishment of the biliary fistula, moderate weakness and atrophy of the skeletal muscles were noted. These progressed gradually during the next 3 months, when the atrophy and paresis were both pronounced. At that time ox bile (75 to 100 cc. daily) was started by mouth and was continued during the last 6 months of this experiment. During this period there was considerable improvement of the muscle strength, but the muscular atrophy persisted. At autopsy, no necrotic muscle lesions could be found, although there was considerable proliferation of subsarcolemmic nuclei. Together with replacement fibrosis this suggested that parenchymatous degeneration had been present earlier. The progress of the dystrophy was arrested apparently by the bile feeding in this animal.

Discussion

The lesions which we have observed in the chronic biliary fistula dogs were of essentially the same type as those described in the nutritional muscular dystrophy of rabbits and guinea pigs by Goettsch and Pappenheimer.[7] Although the diets in their experiments were deficient in vitamin E, addition of wheat germ oil failed to prevent the development of the dystrophy. While Morgulis [8] found that vitamin E is essential for the prevention of the disorder in these animals, he believed that some other factor in addition to vitamin E, perhaps a part of the vitamin B complex, was concerned. Olcott [9] reported dystrophic muscle lesions in suckling rats of vitamin E-deficient mothers, similar to those described by Goettsch and Pappenheimer.[7] Later reports have described the production of the dystrophy in adult rats.[10,11] Knowlton, Hines and Brinkhous [12] have shown that this nutritional dystrophy in rats can be prevented as well as cured by the subcutaneous administration of synthetic vitamin E (α-tocopherol acetate). Thus, from the data available, it is evident that muscu-

lar dystrophy can be produced in a variety of mammals by eliminating vitamin E from an otherwise adequate diet. Since our preliminary report of paralysis in biliary fistula dogs,[2] it has been shown that vitamin E deficiency will cause dystrophy in dogs.[13] Greaves and Schmidt[14] have shown that vitamin E, like many other fat-soluble substances, is poorly absorbed from the intestine unless bile is present, and it seems likely that faulty absorption of vitamin E was responsible for the muscular dystrophy seen in our chronic biliary fistula dogs. Some of the animals with severe dystrophic changes were males, and these showed testicular degeneration of the type described in vitamin E deficiency. That faulty absorption of substances associated with the fats existed in these cases is evident further from the fact that a vitamin K deficiency developed. Also, extensive osteoporosis was present, indicating faulty absorption of vitamin D.

By analogy, it seems likely that this type of muscular dystrophy may be encountered at times in human cases in which there is difficulty in absorbing fat-soluble materials from the intestine. Patients having sprue, for example, are known to develop, at times, an extreme degree of muscular weakness. A muscular dystrophy on the basis of faulty absorption of vitamin E seems, on theoretical grounds, a possible cause for at least part of the muscular weakness.

Summary

A nutritional muscular dystrophy, similar to that which has been produced in several mammals by eliminating vitamin E from the diet, is described in chronic biliary fistula dogs which were maintained on an adequate diet. It is suggested that the dystrophy is due to a vitamin E deficiency which results from faulty absorption in the absence of bile in the intestine.

REFERENCES

1. Smith, H. P., Warner, E. D., Brinkhous, K. M., and Seegers, W. H. Bleeding tendency and prothrombin deficiency in biliary fistula dogs: effect of feeding bile and vitamin K. *J. Exper. Med.*, 1938, **67**, 911–920.
2. Warner, E. D., and Brinkhous, K. M. Muscular dystrophy in biliary fistula dogs. (Abstract.) *Am. J. Path.*, 1939, **15**, 646.
3. Kapsinow, Robert, Engle, Lawrence P., and Harvey, Samuel C. Intra-abdominal biliary exclusion from the intestines—cholecystnephrostomy, a new method. *Surg., Gynec. & Obst.*, 1924, **39**, 62–65.

4. Hawkins, W. B., and Whipple, G. H. Bile fistulas and related abnormalities. Bleeding, osteoporosis, cholelithiasis and duodenal ulcers. *J. Exper. Med.*, 1935, **62**, 599–620.

5. Hawkins, W. B., and Brinkhous, K. M. Prothrombin deficiency the cause of bleeding in bile fistula dogs. *J. Exper. Med.*, 1936, **63**, 795–801.

6. Mason, Karl E. Differences in testis injury and repair after vitamin A-deficiency, vitamin E-deficiency, and inanition. *Am. J. Anat.*, 1933, **52**, 153–239.

7. Goettsch, Marianne, and Pappenheimer, Alwin M. Nutritional muscular dystrophy in the guinea pig and rabbit. *J. Exper. Med.*, 1931, **54**, 145–165.

8. Morgulis, Sergius. Nutritional Muscular Dystrophy. Nutrition XVI, E. F. Terroine, Ed. Hermann & Cie, Paris, 1938.

9. Olcott, H. S. The paralysis in the young of vitamin E deficient female rats. *J. Nutrition*, 1938, **15**, 221–227.

10. Knowlton, G. C., and Hines, H. M. Effect of vitamin E deficient diet upon skeletal muscle. *Proc. Soc. Exper. Biol. & Med.*, 1938, **38**, 665–667.

11. Evans, Herbert M., Emerson, Gladys A., and Telford, Ira R. Degeneration of cross striated musculature in vitamin E-low rats. *Proc. Soc. Exper. Biol. & Med.*, 1938, **38**, 625–627.

12. Knowlton, G. C., Hines, H. M., and Brinkhous, K. M. Cure and prevention of vitamin E-deficient muscular dystrophy with synthetic α-tocopherol acetate. *Proc. Soc. Exper. Biol. & Med.*, 1939, **42**, 804–809.

13. Anderson, H. D., Elvehjem, C. A., and Gonce, J. E., Jr. Vitamin E deficiency in dogs. *Proc. Soc. Exper. Biol. & Med.*, 1939, **42**, 750–755.

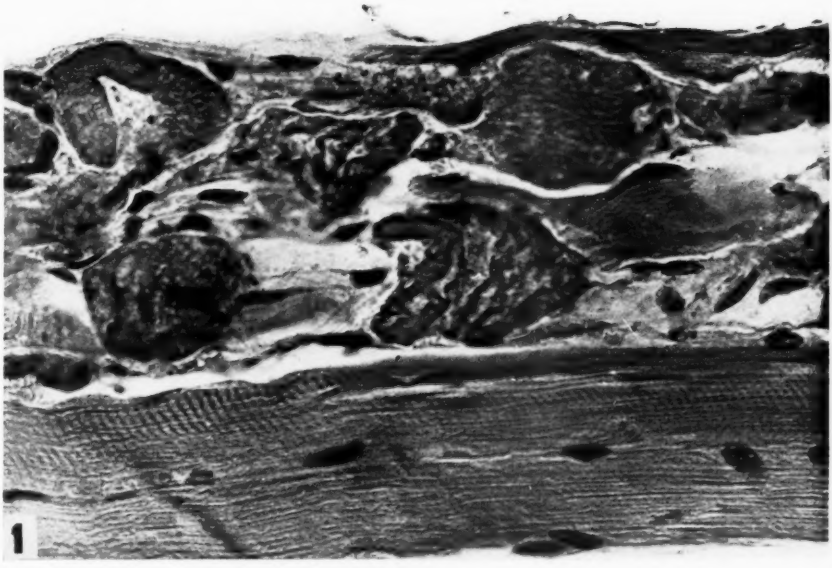
14. Greaves, Joseph D., and Schmidt, Carl L. A. Relation of bile to absorption of vitamin E in the rat. *Proc. Soc. Exper. Biol. & Med.*, 1937, **37**, 40–42.

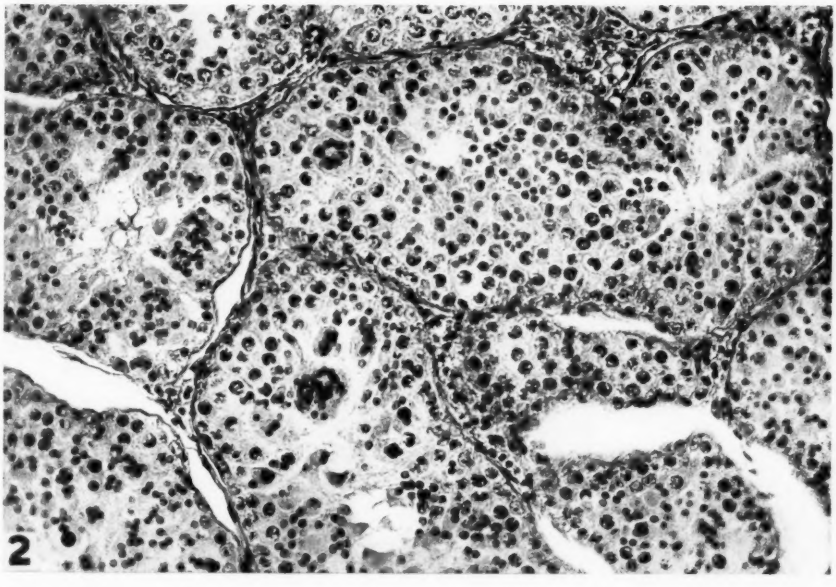
DESCRIPTION OF PLATE

PLATE 17

FIG. 1. Necrosis of voluntary muscle fibers. × 600.

FIG. 2. Testis, showing impaired spermatogenesis. Note multinucleated giant cells. × 220.





Brinkhous and Warner Muscular Dystrophy in Biliary Fistula Dogs

ENDOMETRIAL RESPONSE TO DIETHYLSTILBOESTROL IN RADIUM-INDUCED MENOPAUSE*

ROBERT C. GRAUER, M.D., CHESTER F. BEALL, M.D., AND G. RANDOLPH WILSON, M.D.

(From the Department of Research in Endocrinology and Metabolism of the William H. Singer Memorial Research Laboratory, Allegheny General Hospital, Pittsburgh, Pa.)

It is generally recognized that the menopause syndrome following radium-induced castration results in a clinical condition more difficult to treat than if it results from surgical castration or follows physiological involution. No report was found in which special attention had been paid to the effect of estrogenic substances on the genital tract in patients who had previously been treated with sufficient radium to produce a state of amenorrhea. Hence, it was decided to observe the anatomical changes in the uterus resulting from the use of radium and to study, subsequently, the influence of diethylstilboestrol [1] (4:4-dihydroxy-a:b-diethylstilbene)† at various intervals.

The age of the patients under observation varied from 42 to 50 years at the time of radium insertion. The group consisted of 10 patients who were suffering from bleeding dysfunction incidental to the oncoming menopause. Each had been exposed to 1200 to 1800 mg. hours of radium and one had received 2400 mg. hours of radium irradiation. Diagnostic curettements were done in all cases before radium was inserted in order to eliminate the possibility of the existence of malignancy. This also afforded an opportunity to study the histological appearance of the untreated endometrium. One to 2 years after the radium had been inserted and the bleeding had ceased, attempts were made to secure biopsies. One patient was curetted 5 months after the radium treatment. Vaginal smears were taken in all cases when the endometrial biopsies were secured in order to compare them with smears to be taken at subsequent intervals after treatment with

* Read before the Pittsburgh session of the American Association of Pathologists and Bacteriologists March 22, 1940.

† The stilboestrol used in this study was kindly supplied by Dr. Joseph A. Morrell of E. R. Squibb & Sons.

Received for publication June 8, 1940.

stilboestrol. The patients were then given 3 mg. of stilboestrol by mouth daily for 2 weeks. At the end of the 2 weeks, attempts were made to secure endometrial biopsies at the same time that the vaginal smears were taken. Only 4 patients yielded enough endometrium to be examined microscopically. The treatment was then continued for 5 weeks, until a total of 114 mg. of stilboestrol had been administered to each patient. Diagnostic endometrial biopsies were again attempted on all 10 patients by means of a Meigs' curette and material for study was secured from 8. Following this, the final phase of the study was continued for a period of approximately 4 months, during which time each patient had received a total of 324 mg. of stilboestrol. Vaginal smears were taken at weekly intervals and were evaluated according to the criteria established by Papanicolaou and Shorr.[2]

The experience of others [3] has been that vaginal epithelial and endometrial responses occur after 2 mg. of stilboestrol have been administered daily for a period of 1 to 2 weeks. However, the reports referred to patients with a physiological menopause or with secondary amenorrhea. When our radium-treated cases were examined after 2 weeks, we found that we obtained no endometrial response nor changes in the vaginal smears. At the end of 5 weeks of treatment, a proliferative endometrium was observed and definite vaginal smear responses took place. It will be observed in referring to Table I that 6 cases showed definite proliferative

TABLE I
Biopsies and Vaginal Smears

	Endometrial biopsies					
	Atrophic endometrium	Proliferative endometrium	Secretory endometrium	Cystic glands	None obtained	Vaginal smears
Before radium	0	7	2	1	0	Not taken
After radium	3				7	Menopausal smear
Treatment 2 weeks (48 mg.)	4				6	No response
Treatment 5 weeks (114 mg.)	2	6			2	5 responded

responses of the endometrium after 5 weeks of treatment; 2 showed very slight response, designated as atrophic endometriums, and in 2 cases no biopsy was obtained due to a complete closure of the external os, preventing introduction of a curette.

Of the 8 patients who had patulous cervical canals, 3 (Table II) experienced cyclic bleeding during the course of the study. In all 3 cases the first period of vaginal bleeding occurred 6 weeks after treatment had been initiated. Subsequently, bleeding occurred in 2 cases at monthly intervals. This bleeding cannot be interpreted entirely as a type of withdrawal bleeding, since the treatment was

TABLE II
Bleeding Response to Stilboestrol

	No.	Dose	Treated	Vaginal bleeding
Patients with menopausal syndrome due to radium	10	324 mg.	4 months	3 cases
Patients with physiological menopausal syndrome	7	90–100 mg.	4–6 weeks	6 cases
Patients with surgical menopausal syndrome	11	Bleeding occurred in those patients who had had amputation of the fundus		

continuous, but apparently represented the result of a considerable degree of endometrial proliferation. In contrast to this observation it is interesting to observe that 6 out of 7 patients with a physiological menopause developed vaginal bleeding in the course of treatment. Also all of a group with a surgically-induced menopause in whom only a fundal amputation had been done, showed vaginal bleeding. These latter groups were observed in another study but were compared to the radium-induced menopause cases in order to evaluate the differences in physiological response. When we compare (Table I) the response of the vaginal epithelium, as evidenced by smears, with the changes that occurred in the endometrium, we find that the time required for the vaginal epithelium to respond to stilboestrol parallels that for the endometrial response.

MICROSCOPIC CHANGES

The sections of the endometrium taken from patients in whom bleeding dysfunction occurred before radium was inserted, exhibited a definite morphological character which is seen in bleeding associated with a failing corpus luteum. The glands all presented a marked degree of epithelial proliferation in which the cells were several layers in thickness and which showed the presence of mitotic figures. They were supported by a moderately congested endometrial stroma. Thus they presented the dual picture of endometrial glands during the follicular phase and an edematous, congested stroma similar to that seen in the progesta-

tional phase. Only 2 cases showed tortuous glands in which secretory vacuoles were present; yet even these showed an intermingling with the changes of a proliferative phase—evidence of a failing corpus luteum. One patient showed a moderate degree of cystic distention approximating the Swiss cheese type of endometrium, while 7 cases showed a proliferative endometrium with no evidence of progestational effect on the glands.

After radium application, attempts were made to secure biopsies and in only 3 instances did successful curettements result. These showed an atrophic endometrium in which a few isolated glands were supported by a hyalinized stroma. In 7 cases no material was obtainable. After 5 weeks of treatment, 6 successful biopsies were obtained. These tended to duplicate the picture which was observed with the original curettements before radium was inserted. The glands were proliferative, the stroma showed congestion and edema, and the stroma cells were proliferative and hypertrophied. In a few cases some myometrium was secured and it was observed that the myometrial cells were hypertrophied and stimulated to proliferation.

It was extremely interesting to observe the type of reaction that was secured by stimulation with stilboestrol in consequence of the previous treatment with radium. If only vestiges of endometrial acini remained, the stilboestrol was sufficient to stimulate them to proliferation. The wall of the uterus in some of the cases showed dense hyalinization which did not respond to the stilboestrol but contained contiguous stimulated myometrium or endometrial glands or stroma. There were areas in which isolated muscle fibers had not been destroyed and were brought to life, so to speak, in the interstices of the hyalinized masses. In 1 case it was observed that the stroma cells of the endometrium had begun to be stimulated in 2 weeks, while in 5 weeks they showed approximation of the premenopausal state. This case demonstrated the earliest stimulation observed in the group studied.

Discussion

Our studies, made on a series of cases in which an artificial menopause had been produced by radium, confirmed the belief that such cases are more refractory to stilboestrol than cases of surgical sterilization or those associated with normal physiological

processes. In the radium-treated cases we were dealing with the dual effects on the endometrium of the physiological regression incidental to menopause age, plus the destructive effects of radium. In the physiological menopausal cases the endometrium remained intact even though it became atrophic. The same consideration holds for whatever endometrium is left following partial hysterectomy, which may accompany ovariectomy. The response appeared to be related quantitatively to the intact endometrium that persisted.

The efficacy of radium or X-ray irradiation in bringing about a prompt amenorrhea in these cases can be explained when it is recalled that the irradiation acts on the nucleus of the cell and not on the cytoplasm. The normal cell is more vulnerable during the time of cell division than during the resting stage. Since patients with bleeding dysfunction exhibit a proliferative endometrium, in which there are numerous mitotic nuclei, the cells are extremely vulnerable to irradiation. In addition to bringing about a prompt cessation of bleeding, radium also produces a widespread destruction of cells. In the bleeding dysfunction cases that we studied, the majority (70 per cent) showed a proliferative endometrium in which there were numerous mitotic figures. Marked destruction of the glandular structures resulted as a consequence of the irradiation. The supportive tissue is more resistant to irradiation than glandular tissue and, consequently, this could be stimulated somewhat earlier than could the acini. This was made evident by the case which showed an early stromal response after 2 weeks of treatment. The only evident explanation of the occurrence of bleeding in only three cases under treatment, as compared to the much higher percentage in the physiological cases, is that endometrial stimulation by stilboestrol is inhibited in the radium-treated cases to a greater degree than in those not irradiated.

Summary

1. Diethylstilboestrol stimulates the glands, the stroma and the myometrium in radium-treated cases of bleeding dysfunction.

2. The endometrial and vaginal response to stilboestrol in radium-treated cases is delayed, and the dose required is greater, as compared to cases of physiological menopause. The stilboestrol

tends to produce a proliferative endometrium and stroma which duplicates the appearance of the endometrium before radium was inserted.

3. There is less tendency to produce bleeding in the radium-treated menopausal cases than in others.

4. Radium injures the stroma and myometrium as well as the glands. Stimulation by stilboestrol affects all three components.

REFERENCES

1. Dodds, E. C., Goldberg, L., Lawson, W., and Robinson, R. Oestrogenic activity of certain synthetic compounds. *Nature*, 1938, **141**, 247–248.

2. Papanicolaou, George N., and Shorr, Ephraim. The action of ovarian follicular hormone in the menopause, as indicated by vaginal smears. *Am. J. Obst. & Gynec.*, 1936, **31**, 806–831.

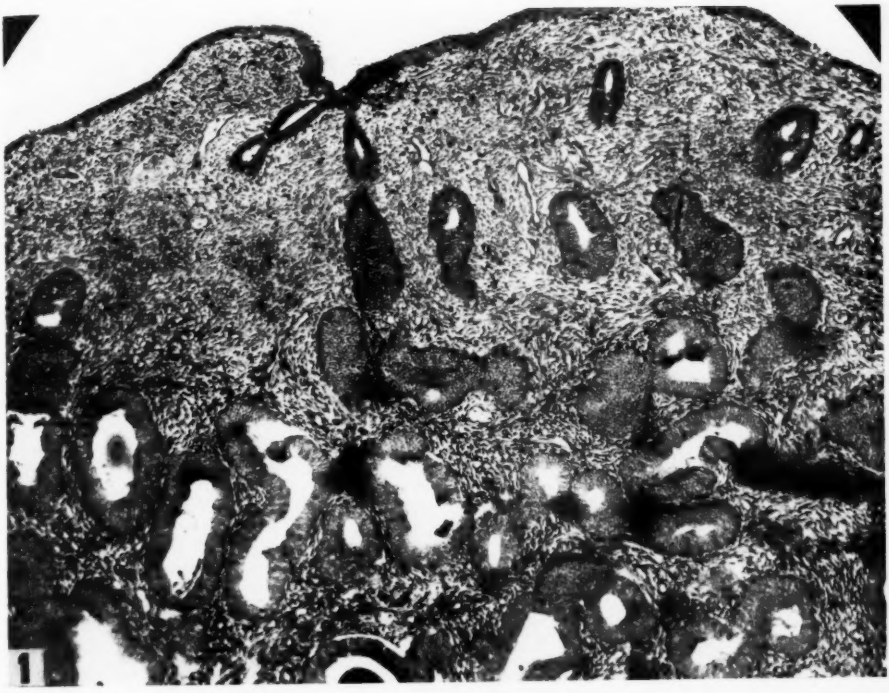
3. Winterton, W. R., and MacGregor, T. N. Clinical observations with stilboestrol (diethylstilboestrol). *Brit. M. J.*, 1939, **1**, 10–12.

DESCRIPTION OF PLATES

PLATE 18

FIG. 1. Proliferative congested endometrium of a bleeding dysfunction case before radium treatment. × 100.

FIG. 2. Endometrial response after 5 weeks' administration of stilboestrol 2 years after radium-produced atrophy. Had four periods of cyclic bleeding. × 100.


1

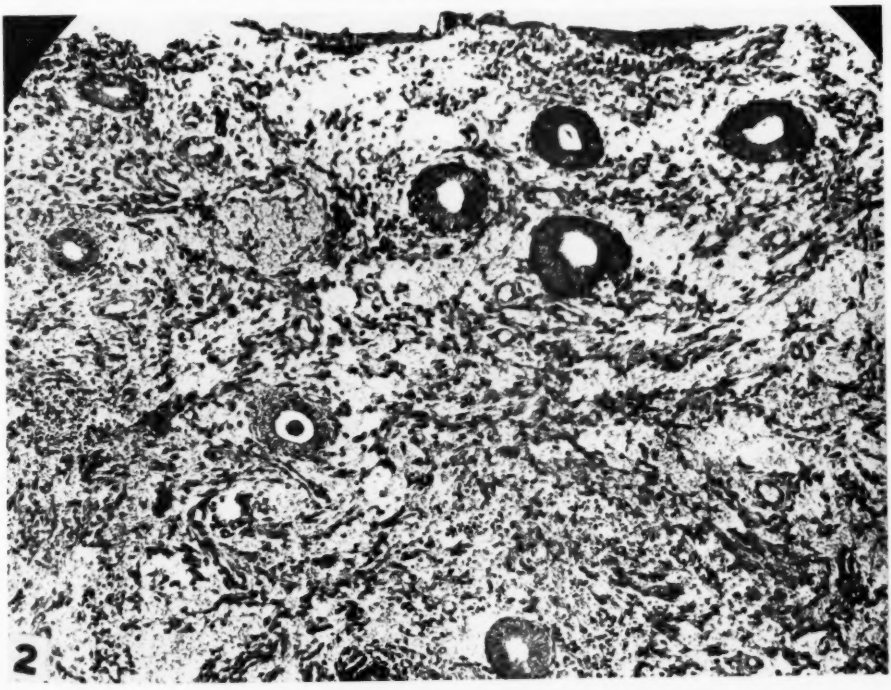
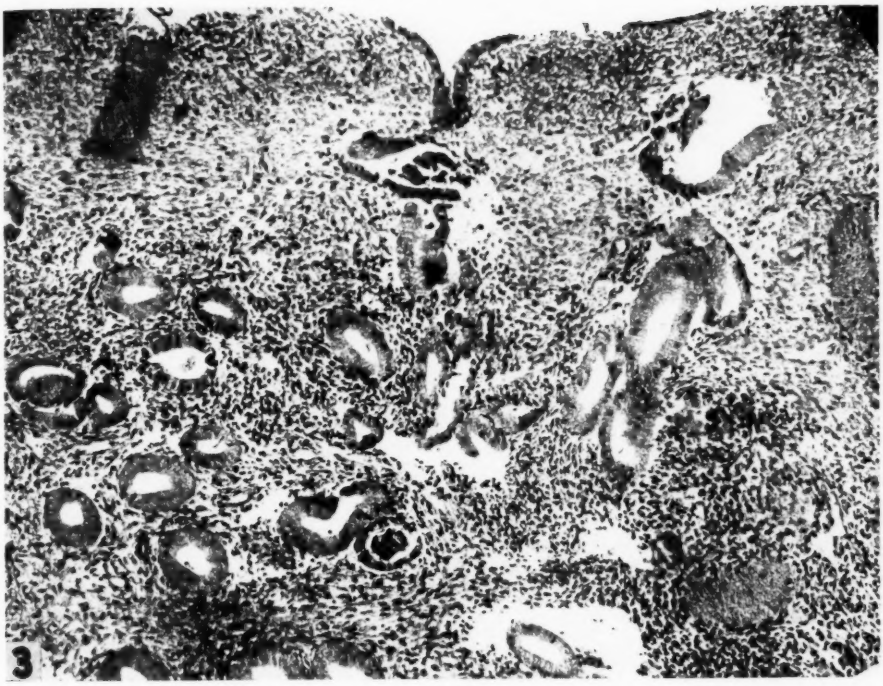

2

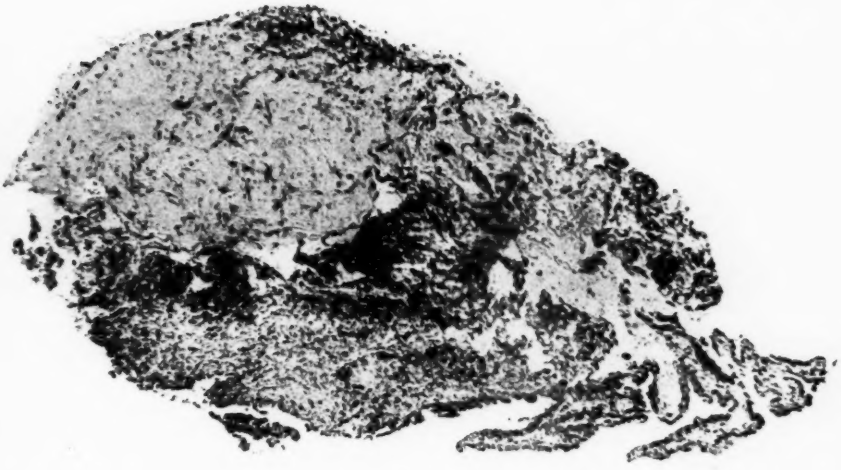
PLATE 19

FIG. 3. Proliferative endometrium associated with continuous menorrhagia. No progestational effect. × 100.

FIG. 4. After 5 weeks' treatment with stilboestrol. Stimulated cells seen in interstices of hyalinized myometrium in upper left. Glandular epithelial stimulation in upper right. No cyclic bleeding occurred. × 46.



3



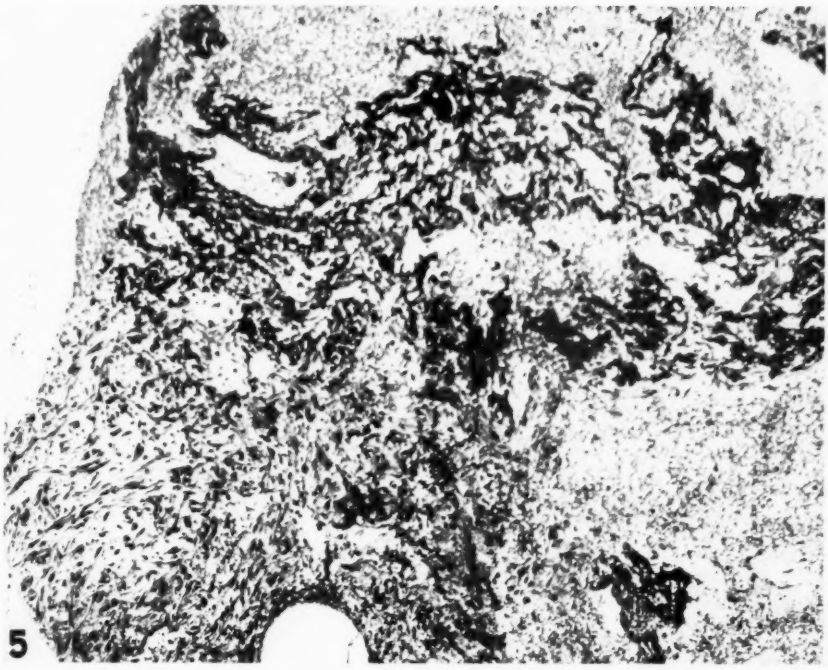
4

Grauer, Beall and Wilson Endometrial Response to Diethylstilboestrol

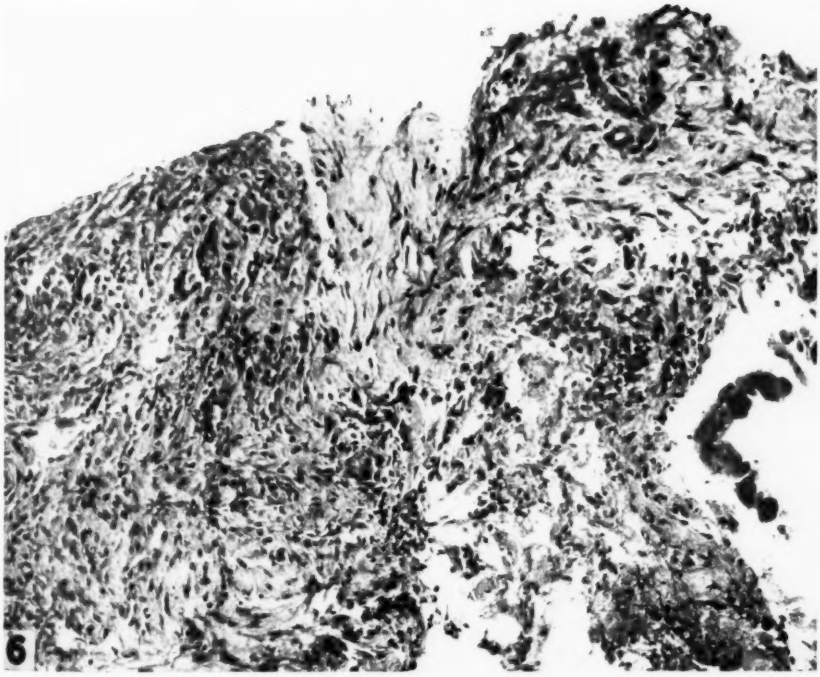
PLATE 20

FIG. 5. Stimulation of stroma cells after 2 weeks' administration of stilboestrol. Earliest evidence of stilboestrol effect. × 100.

FIG. 6. Same case as Figure 1 after 5 weeks' administration of stilboestrol. Note glandular as well as stroma cell proliferation. × 200.



5



6

Grauer, Beall and Wilson Endometrial Response to Diethylstilboestrol

EFFECT OF DIBENZANTHRACENE ON TRANSPLANTABLE MAMMARY ADENOFIBROMA OF THE WHITE RAT *

J. H. Davis, M.D., K. M. Murphy, M.D., and L. A. Emge, M.D.

(From the Department of Obstetrics and Gynecology, Stanford University School of Medicine, San Francisco, Calif.)

Since 1928 we have been engaged in the study of growth behavior of mammary adenofibromas of white rats in relation to various phenomena of functional degeneration. Our main approach to the problem has been through serial transplantation of tumor tissue into a highly inbred strain of white rats originally derived from the Wistar stock. We reported elsewhere [1] that in certain instances it is possible to maintain the morphology and growth behavior of the original tumors through many transplantations but that, for reasons yet unknown, certain lines of transplanted adenofibromas change fairly abruptly into fibromas and sarcomas. This latter change offers an interesting problem in spontaneous malignant degeneration. The transition is invariably initiated by the gradual loss of glandular elements. In due time a pure connective tissue tumor forms, and ultimately differentiates into either a very mature fibroma or a viciously growing sarcoma. In isolated instances adenofibromas have assumed the morphologic appearance of adenocarcinoma but further transplantations have failed to prove functional malignancy. So far, all attempts to induce malignancy in adenofibromas by other means, such as rapid and frequent breeding or overdosing with growth hormone or huge doses of estrogenic substances, have been unsuccessful.[2] It therefore seems unlikely that the sarcomatous degeneration taking place during transplantation of our tumors can be explained on an endocrine basis. Neither do we think that the mere mechanical transference of tumor tissue from one host to another is the explanation. If it were, all adenofibromatous tissue transplanted by us would in time have changed into sarcoma, which has not been true. Some of our adenofibromatous tumor material has survived many transplantations since 1928 and still is morphologically and functionally similar to the original tumor. There must be

* Supported by a grant from Rockefeller Fluid Research Fund of Stanford University School of Medicine.

Received for publication May 27, 1940.

another reason, therefore, for tumor mutation, and the experiments reported here represent one of our attempts to obtain information in this direction.

We are reporting two phases of our study: (1) an attempt to determine the susceptibility of our strain of rats to 1:2:5:6 dibenzanthracene; and (2) an attempt to determine whether such an agent is capable of inducing changes in implanted adenofibromas similar to those occurring spontaneously in the course of continued transplantations.

To our knowledge, the strain of rats used by us never has produced spontaneous sarcoma or carcinoma. We have stated elsewhere [2] that we believe that in this strain both mammary and genital tissues exposed to superphysiologic estrogenic stimulation are inherently protected against malignant degeneration.

Experiment I

The Susceptibility of Our Rats to Dibenzanthracene

To 37 young unimplanted male and female rats were given three or four injections of 1:2:5:6 dibenzanthracene at weekly intervals.* Twelve rats survived 100 days or more. Ten of these developed tumors at the site of injection (Table I). While the series lacked sufficient size and uniformity to allow any statistical deductions, it is apparent that dibenzanthracene is able to induce a high percentage of local sarcomas in our rats.

The sarcomas thus produced presented a wide range in time of appearance, 102 days being the shortest and 236 days the longest

Table I
Occurrence of Induced Sarcomas in Unimplanted Animals Treated with Dibenzanthracene

No. of Animals	Age at injection	No. living 100 days	No. of induced tumors	Days to Appearance	Appearance to removal	Tumor weight
	days				*days*	*grams*
A. Unimplanted males						
11	34	2	1	199	34	56.0
20	102 to 156	9	8	102 to 220	2 to 52	0.7 to 56.3
B. Unimplanted females						
	days				*days*	*grams*
6	96 to 105	1	1	236	25	30.0

* Injections of 0.5 to 1.0 cc. of 0.4 per cent dibenzanthracene (E. K. Co.) in lard were given subcutaneously into the loose connective tissue beneath the shoulder, remaining well away from the site of the implanted tumor. Previous trials with intraperitoneal injections proved this method to be undesirable because of the markedly increased hazard due to toxicity.

period. Once established, these tumors grew very rapidly and infiltrated muscle and surrounding tissue. Their rate of growth was similar to that of our sarcoma E I–2,[1] but differed from it by a greater tendency to invade. Microscopically, they differed in degree of anaplasia and mitotic activities, both of which are considerably greater in the chemically induced tumors (Figs. 1 and 2; 3 and 4). The dibenzanthracene tumors were as readily transplantable into other strain-related rats as our sarcomas. Serial transplantation did not alter their malignant behavior. They failed to produce distant metastases and in all instances occurred only at the site of injection. In this respect they resembled our sarcomas and, like them, killed by some unknown mechanism not dependent on size of metastatic growth.

TABLE II

Adenofibroma Growth, Body Weight Changes and Occurrence of Induced Sarcoma in Control and 1:2:5:6 Dibenzanthracene Groups

Female Series

Treated Group

Animal No.	Body weight gain per day	Implanted tumor		Induced sarcoma		
		Final weight	Gain per day	Injection to appearance	Appearance to removal	Weight on removal
	grams	*grams*	*grams*	*days*	*days*	*grams*
1572	0.32	24.33	0.180	173	29	60.00
1578	.11	79.00	.416	180	60	43.98
1592	−.18	50.09	.371	—	—	—
1615	.20	38.08	.280	125	36	64.20
1563	.25	22.48	.223	—	—	—
1565	.17	51.00	.375	271	36	90.00
1576	.15	41.00	.304	271	36	67.68
1613	.24	35.80	.188	292	15	7.80
Average	0.16		0.292	219		

Control Group

Animal No.	Body weight gain per day	Implanted tumor	
		Final weight	gain per day
	grams	*grams*	*grams*
1551	0.19	60.10	0.445
1564	.37	27.46	.272
1568	.16	—	—
1571	.24	93.43	.692
1573	.21	41.11	.305
1577	.07	93.80	.695
1594	.15	62.00	.614
1612	.33	26.60	.196
1614	.40	48.24	.478
1567	.07	93.50	.926
1569	.23	—	—
Average	0.22		0.514

TABLE III
Male Series
Treated Group

Animal No.	Body weight gain per day	Implanted tumor		Induced sarcoma		
		Final weight	Gain per day	Injection to appearance	Appearance to removal	Weight on removal
	grams	*grams*	*grams*	*days*	*days*	*grams*
1600	0.19	32.36	0.140	180	22	46.00
1610	.05	—	—	152	36	108.75
1618	.60	2.40	.010	194	8	18.08
1591	.63	12.70	.059	152	36	39.00
1602	.46	4.24	.018	—	—	—
1604	.69	7.00	.030	229	35	106.32
1606	.55	5.81	.025	243	23	37.10
1608	.53	4.33	.019	215	4	2.84
Average	0.46		0.044	195		

Control Group

Animal No.	Body weight gain per day	Implanted tumor	
		Final weight	Gain per day
	grams	*grams*	*grams*
1599	0.63	9.40	0.041
1601	.73	9.25	.040
1603	.51	2.03	.009
1605	.71	7.10	.031
1607	.58	6.16	.027
1609	.58	41.50	.180
Average	0.62		0.055

EXPERIMENT II

The Effect of a Cancerigenic Agent on the Behavior of a Benign Transplantable Adenofibroma

A richly glandular adenofibroma (Fig. 5) was implanted into 34 rats (19 females and 15 males) approximately 90 days old. One month later, after the implants were well established, 8 males and 8 females were given injections of 0.4 per cent dibenzanthracene exactly as described in Experiment I. The remaining implanted animals were set aside for controls. (One male died 52 days after implantation and was excluded.) Results are given in Tables II and III. The figures indicate that the growth of the implanted tumors in the treated males did not differ significantly from that in the untreated males. However, there was a definite retardation of growth in the treated females as compared with the controls (Table IV).

TABLE IV
Mean Daily Changes in Tumor Weight (Gain per Day)

	Controls	Treated	Difference	P*
Males	.055	.043	.012	.7
Females	.514	.292	.222	.027

* P = probability of difference as great or greater than that obtained by chance alone. See Fisher, R. A. Statistical Methods for Research Workers. Oliver and Boyd, London, 1934, Ed. 5, Chap. 5.

From long experience with these transplantable adenofibromas we know that the males commonly produce a tumor rich in fibrous constituents and poor in glandular components, while the females usually produce the opposite type. These characteristics evidently were not disturbed in the treated groups (Figs. 6–9). In both female groups the usual whorled pattern of an adenofibroma rich in glandular elements was maintained, and in none was there any evidence of unusual activity or of glandular or connective tissue disorganization.

The sarcomas developing at the site of dibenzanthracene injection were of the same character and occurred with the same frequency as those described in Experiment I. The interval between initial injection and first appearance of the tumor varied from 125 to 292 days. Tumors occurred in 7 of the 8 treated males and 6 of the 8 females. Microscopically, the sarcomas were identical with those of Experiment I.

DISCUSSION

Regardless of the presence of a benign implanted tumor, 1:2:5:6 dibenzanthracene induced local malignancies to the extent of over 80 per cent in our strain of rats. Other workers have obtained a wide range of difference in the susceptibility of different strains of rats to dibenzanthracene. Burrows, Hieger and Kennaway [3] in 1932 found 15 tumors in 67 rats (22 per cent); Barry and Cook [4] found 8 tumors in 20 rats (40 per cent); Shear [5] in 1936 reported 11 tumors in a group of 18 rats (61 per cent); and Haagensen and Krehbiel [6] found 26 tumors in 128 rats (20 per cent). A similar variation in the susceptibility of mice has been reported.

The susceptibility of rats of our strain to this carcinogenic agent is in marked contrast to their inherent resistance to spon-

taneous tumor formation. The frequency of spontaneous mammary adenofibromas has been but 0.3 per cent. Spontaneous malignant tumors have never been seen in this strain of rats.

The high incidence of chemically induced tumors indicates that our strain of rats is not completely refractory to malignant degeneration. But whatever this peculiarity may be, it seems to be confined strictly to local tissue reactions. In some ways this parallels the behavior of the tissues of our tumors. However, we do not believe that a mechanism as simple as a specific chemical stimulation is responsible for the sarcomatous changes occurring in our tumors. That the susceptibility to coal tar tumors does not necessarily bear a relationship to susceptibility to spontaneous tumors or tumor transplantability was shown by Andervont [7] for mice.

The results of our second experiment further emphasize the remoteness of the possibility of a chemical agent causing malignant changes in transplantable mammary adenofibromas. Beyond depressing the rate of tumor growth in females, dibenzanthracene failed to induce morphologic changes in these tumors when injected at a distance. The toxicity of the chemical agent may have been concerned with the decrease in growth by disturbing body metabolism, as pointed out by Lees,[8] but since this phenomenon was peculiar to females this cannot be the sole explanation. Our observations differ from those of others in the type of tumor used. It is evident that the influence of dibenzanthracene on a slowly growing adenofibroma may differ from that on the rapidly growing malignant tumors used by others because of the longer duration of exposure.

The effect of dibenzanthracene on tumor-bearing animals has been studied by several investigators. Haddow,[9] in studying this relation to the Jensen rat sarcoma, found it to inhibit tumor growth. In other investigations [10-14] these findings have been confirmed for various transplantable tumors in the rat. In mice, there are differences in susceptibility to cancerigenic hydrocarbons. Pybus and Miller [15] and Haddow [16] reported an inhibitory effect similar to that observed in rats, while Andervont [17] noted an increased incidence of lung carcinoma in a strain of mice subject to spontaneous lung cancer. Taschner, Gottlieb, Spritzer and Plonskier [18] reported a higher percentage of takes of Jensen rat

sarcoma in mice treated with methylcholanthrene. Appel, Strauss, Kolischer and Necheles [19] found that dibenzanthracene produced a more rapid growth of the Brown-Pearce carcinoma of rabbits, with an increase in metastatic invasion. Rabbits and mice, therefore, may differ from rats in degrees of susceptibility to chemically induced tumors. Our findings in a cancer-free strain of rats prove that the inherent protection against malignancy can be overcome to some extent but that such response is confined to tissues in contact with the agent, since implanted adenofibromas fail to undergo malignant change, although capable of doing so spontaneously.

In comparing the sarcomas induced by dibenzanthracene with those occurring in the course of transplantation of our adenofibromas it is evident that the former possess a greater degree of malignancy. However, they resemble each other in that they fail to produce distant metastases, recur after removal and yield 100 per cent takes upon subsequent transplantation. The malignant degenerative processes of the two, on the whole, are similar, but in view of the lack of influence of dibenzanthracene on the implanted benign tumors it is not likely that a cancerigenic agent of the dibenzanthracene type is the factor causing sarcomatous degeneration of the benign mammary adenofibromas transplanted by us into a strain of rats refractory to spontaneous cancer.

Conclusions

1. The subcutaneous injection of 1:2:5:6 dibenzanthracene in a strain of rats refractory to spontaneous cancer produces sarcomas in over 80 per cent of animals surviving the injection period 100 days or more.

2. The sarcoma induced by dibenzanthracene is readily transplantable through six generations, fails to metastasize, and microscopically is more anaplastic than sarcomas derived from the adenofibromas.

3. The presence of a previously implanted benign tumor has no effect on the incidence or character of these induced sarcomas.

4. Dibenzanthracene fails to induce malignant changes in a transplantable mammary adenofibroma known to undergo spontaneous malignant degeneration.

5. Dibenzanthracene exerts an inhibitory effect on the growth of adenofibromas in the female rats.

6. It is not likely that spontaneous sarcomatous degeneration of transplanted mammary rat adenofibromas is the result of a chemical cancerigenic agent of the dibenzanthracene type.

REFERENCES

1. Emge, Ludwig A. Sarcomatous degeneration of transplantable mammary adenofibroma of the white rat. *Arch. Path.*, 1938, **26**, 429–440.
2. Emge, Ludwig A. Estrogenic hormones and carcinogenesis. *Surg., Gynec. & Obst.*, 1939, **68**, 472–480.
3. Burrows, H., Hieger, I., and Kennaway, E. L. The experimental production of tumours of connective tissue. *Am. J. Cancer*, 1932, **16**, 57–67.
4. Barry, G., and Cook, J. W. A comparison of the action of some polycyclic aromatic hydrocarbons in producing tumours of connective tissue. *Am. J. Cancer*, 1934, **20**, 58–69.
5. Shear, M. J. Studies in carcinogenesis. I. The production of tumors in mice with hydrocarbons. *Am. J. Cancer*, 1936, **26**, 322–332.
6. Haagensen, Cushman D., and Krehbiel, Otto F. The morphology of the sarcomas produced by 1:2:5:6-dibenzanthracene. *Am. J. Cancer*, 1936, **26**, 368–377.
7. Andervont, H. B. Susceptibility of mice to spontaneous, induced, and transplantable tumors. A comparative study of eight strains. *Pub. Health Rep.*, 1938, **53**, 1647–1665.
8. Lees, J. C. Tumour-inhibiting properties of 1:2:5:6 dibenzanthracene. *Quart. J. Exper. Physiol.*, 1937, **27**, 181–191.
9. Haddow, Alexander. Influence of certain polycyclic hydrocarbons on the growth of the Jensen rat sarcoma. *Nature*, 1935, **136**, 868–869.
10. Haddow, A., and Robinson, A. M. The influence of various polycyclic hydrocarbons on the growth rate of transplantable tumours. *Proc. Roy. Soc., London*, 1937, series B, **122**, 442–476.
11. Pollia, Joseph A. The effect of lard oil, sesame oil, acacia, retene, and 1:2:5:6 dibenzanthracene on certain organs and a transplantable rat sarcoma in animals of pure breed. *Radiology*, 1937, **29**, 683–694.
12. McJunkin, Frank A., and Wolavka, William. Effect of 1, 2, 5, 6-dibenzanthracene on spindle cell sarcoma of a rat. *Arch. Path.*, 1938, **25**, 506–513.
13. Haddow, Alexander. The influence of carcinogenic substances on sarcomata induced by the same and other compounds. *J. Path. & Bact.*, 1938, **47**, 581–591.

14. Cook, J. W., and Kennaway, E. L. Chemical compounds as carcinogenic agents. First supplementary report: literature of 1937. *Am. J. Cancer,* 1938, **33**, 50–97.
15. Pybus, F. C., and Miller, E. W. On the effect of 1:2:5:6-dibenzanthracene on spontaneous mouse tumours. *Brit. J. Exper. Path.*, 1937, **18**, 126–137.
16. Haddow, Alexander. The influence of carcinogenic compounds and related substances on the rate of growth of spontaneous tumours of the mouse. *J. Path. & Bact.*, 1938, **47**, 567–579.
17. Andervont, H. B. Further studies on the production of dibenzanthracene tumors in pure strain and stock mice. *Pub. Health Rep.*, 1935, **50**, 1211–1217.
18. Taschner, E., Gottlieb, G., Spritzer, M., and Plonskier, M. La transplantation du sarcome du rat sur des souris préalablement traitées avec une substance cancérigène. *Compt. rend. Soc. de biol.*, 1937, **125**, 10–11.
19. Appel, Max, Strauss, Alfred A., Kolischer, G., and Necheles, H. The effect of 1:2:5:6-dibenzanthracene on the growth of Brown-Pearce rabbit carcinoma. *Am. J. Cancer,* 1938, **33**, 239–245.

DESCRIPTION OF PLATES

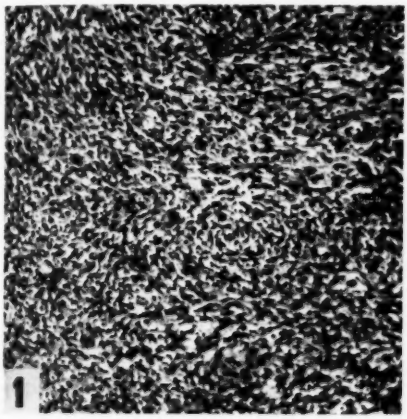
PLATE 21

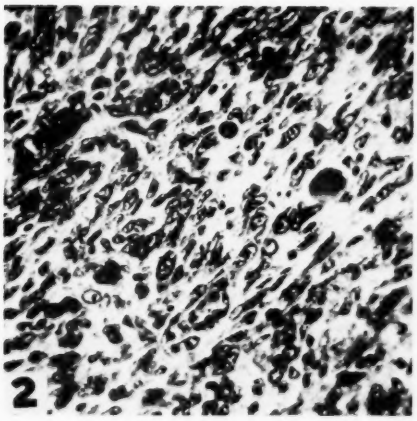
FIG. 1. Dibenzanthracene induced sarcoma (low power). × 100.

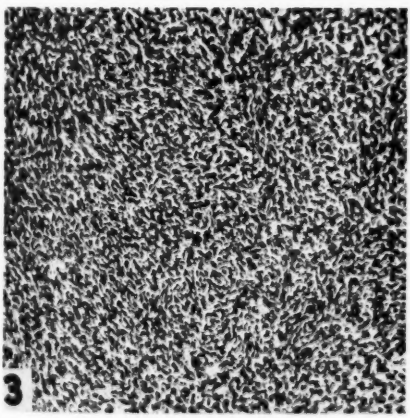
FIG. 2. Dibenzanthracene induced sarcoma (high power). × 450.

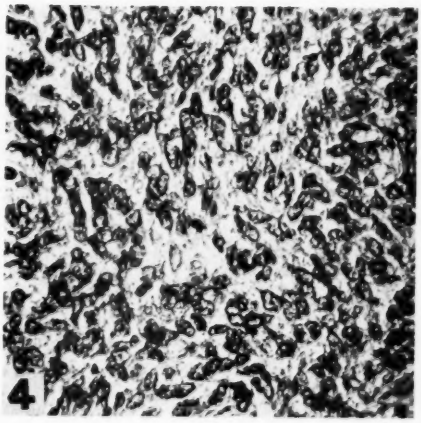
FIG. 3. Sarcoma E I–2 (low power). × 100.

FIG. 4. Sarcoma E I–2 (high power). × 450.









Davis, Murphy and Emge

Dibenzanthracene on Mammary Adenofibroma

PLATE 22

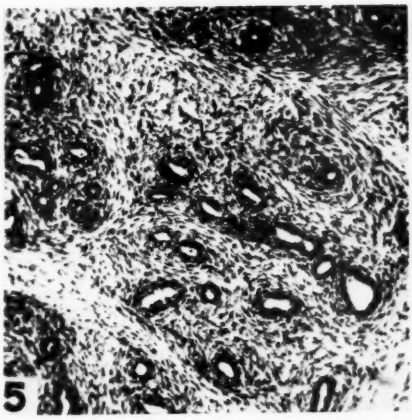
FIG. 5. Adenofibroma, donor to Experiment II. × 100.

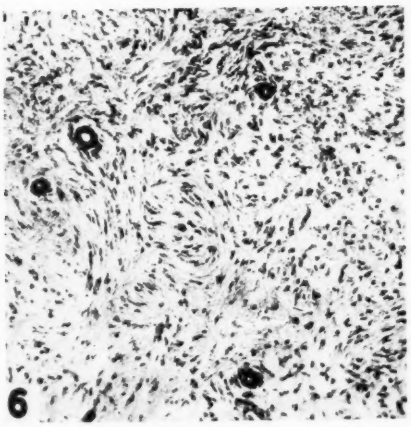
FIG. 6. Fibro-adenoma in an untreated male. × 100.

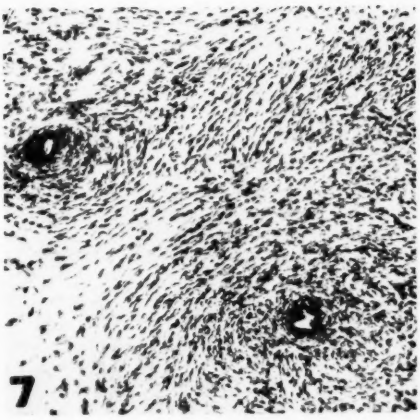
FIG. 7. Fibro-adenoma in a dibenzanthracene treated male. × 100.

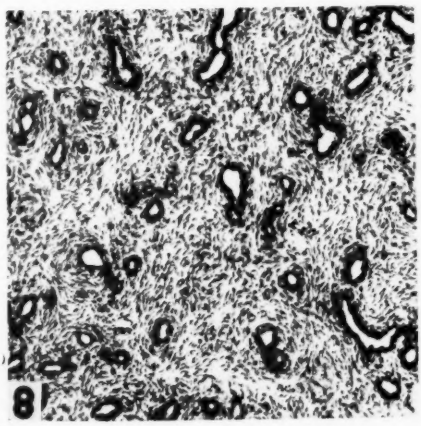
FIG. 8. Adenofibroma in an untreated female. × 100.

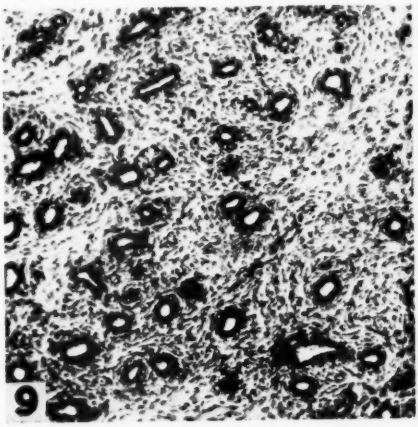
FIG. 9. Adenofibroma in a dibenzanthracene treated female. × 100.











Davis, Murphy and Emge

Dibenzanthracene on Mammary Adenofibroma

THE CHORIO-ALLANTOIC MEMBRANE OF THE DEVELOPING CHICK AS A MEDIUM FOR THE CULTIVATION AND HISTOPATHOLOGIC STUDY OF PATHOGENIC FUNGI *

MORRIS MOORE, Ph.D.

(From the Departments of Mycology and Dermatology of the Barnard Free Skin and Cancer Hospital, St. Louis, Mo.)

In the search for ways to hasten diagnostic procedures and to improve therapeutic measures, microbiologists are employing unprecedented means of biologic investigation. One such method is the use of the chorio-allantoic membrane of the developing chick for the growth and determination of various microörganisms, including numerous viruses, Rickettsiae, bacteria and spirochetes. The use of this structure in the fertile egg for the isolation, identification and further study of organisms has not, in any great measure, been used for the study of fungi, except as mentioned by Goodpasture [1] in explaining the application of the membrane in investigating infectious processes and in examining the fungus-contaminated chorio-allantois.

Egg contents have been employed as enriching substances for cultivation for many years, chiefly in bacteriology and only to a very limited extent in mycology. One of the earliest uses of the egg as a medium for the cultivation of fungi was that of Wolff and Israel [2] who, in 1891, obtained pure cultures of Actinomyces by the inoculation of pus from a retromaxillary nodule into raw and partially boiled hen's and pigeon's eggs.

The use of fertile eggs, however, seems to have had its start with the work of Levaditi [3] in 1906, who infected chick embryos with a spirillum of fowls in continuation of some experiments started by Borrel in 1905. In addition to making other important observations, Levaditi pointed out the significant relationship between living embryonic tissue and susceptibility to infection as found in spirillosis. Later, in 1911, Rous and Murphy,[4] in studying a neoplasm of chickens (Rous sarcoma) which had been discovered by Rous in 1911, were able to demonstrate very success-

* Read at the Third International Congress of Microbiology, Section VI, New York, N.Y., September 4, 1939.

Received for publication May 27, 1940.

fully the value of the chorio-allantoic membrane of fertile eggs as a medium for experimental pathologic problems, emphasizing in particular "the conception of organic differentiation" and "the selective influence of living cells in certain infective processes" as characterized by Goodpasture.[1]

The method of fertile egg inoculation as used today, however, may be chiefly attributed to the unceasing efforts of Goodpasture and his associates. Adapting the technic described by Clark [5] in 1920, Woodruff and Goodpasture [6] in 1931 obtained characteristic histopathologic lesions with fowlpox virus on the chorio-allantoic membranes. Following this initial paper, Goodpasture, in collaboration with various members of his department, further developed the practicability of the technic to a point of reliable experimental procedure, especially as improved by Buddingh.[7] The experiments, thus started, were extended to include viruses (as shown also by Burnet [8] in a number of instances), bacteria, Rickettsiae, and a spirochete,[9] and in June 1939 [10] there appeared a preliminary report on the successful inoculation and "take" with fungi.

Technic

As employed here, the method is essentially the same as that used by Goodpasture and Buddingh [7] and by Burnet [8] for cultivating viruses in the chorio-allantoic membrane of the chick embryo. The coverslip method was employed in preference to the shell-flap method. The former is best adapted for the study of developing lesions.

Because of the slowness of development of fungi, it was found advisable to use eggs incubated 10 to 14 days, as advocated by Goodpasture,[1] depending on the type of organism to be used. Yeastlike organisms show a development time of approximately 5 to 7 days, whereas filamentous forms have a longer evolution time, varying from 5 to 11 days. For such, therefore, it was necessary to use eggs incubated only 10 days.

After inoculation, the eggs were placed in a regular bacteriologic incubator and maintained at a constant temperature without turning. For fungi it was found best to maintain the temperature at approximately 33° C. The eggs were examined daily and, when growth had developed on the membrane for a sufficient period, the

shell was cut just below the surface of the chorio-allantois and removed. A small amount of Zenker's fixative (with 5 per cent glacial acetic acid) was dropped on the inoculated area to harden the membrane, which was then removed with a pair of fine scissors with curved points and placed in Zenker's solution. The membranes were then embedded in paraffin, sectioned and stained with Loeffler's methylene blue and eosin, cleared in xylol and mounted on slides.

Materials*

The organisms cultured and the diseases which they produce are listed as follows in the order of presentation:

I. Superficial dermatomycoses (superficial desquamation, pityriasis)
   A. *Malassezia furfur* (pityriasis or tinea versicolor)
   B. *Pityrosporum ovale* (seborrheic dermatitis)

II. Dermatomycoses (epidermic and dermic lesions)
   A. *Trichophyton gypseum* (trichophytosis, tinea, ringworm)
   B. *Epidermophyton inguinale* (epidermophytosis, athlete's foot, tinea cruris)
   C. *Achorion Schoenleini* (favus)
   D. *Microsporum canis* (microsporosis)

III. Cutaneous, subcutaneous, mucous membrane and internal organ involvement (localized cutaneous lesions, granulomata, deep-seated ulcerative lesions, mucous membrane plaques, lymph stream invasion with dermic and subsequent epidermic involvement, and visceral or generalized diseases)
   A. *Monilia albicans* (moniliasis)
   B. *Geotrichum versiforme* (geotrichosis, mucous membrane and bronchial lesions)
   C. *Zymonema dermatitidis* (blastomycosis)
   D. *Cryptococcus hominis* (cryptococcosis, torulosis)
   E. *Coccidioides immitis* (coccidioidal granuloma)
   F. *Sporotrichum Schencki* (sporotrichosis)

* The binomial nomenclature of the fungi used in this work follows that of Dodge, Carroll William. Medical Mycology, Fungous Diseases of Men and Other Mammals. C. V. Mosby Company, St. Louis, 1935.

G. *Actinomyces bicolor* (actinomycosis)
H. *Monosporium apiospermum* (maduromycosis, Madura foot)
I. *Phialophora verrucosa* (chromomycosis)

When possible, young subcultures of several days' growth were used in order to obtain active proliferation. Several of the organisms had been isolated from human lesions only a short time prior to experimentation.

Lesions of the Chorio-allantoic Membrane

I. Superficial dermatomycoses (superficial desquamation, pityriasis)

A. *Malassezia furfur* (Robin) Baillon, 1889.

Eggs were incubated 11 days and observed 5 days after inoculation. Macroscopically, the lesion on the chorio-allantoic membrane, including the inoculum, had the appearance of a nodule, somewhat irregular in outline. There was a slight opacity in the membrane around the periphery of the nodule. The embryos were alive.

Microscopically, a section of the membrane through the nodule showed it to be made up of the inoculum of fungus. Underlying the fungous growth and fading into the ectoderm was a translucent, more or less granular, substance which stained with eosin. At the junction of this layer and the ectoderm there was a heavy infiltration of monocytes, indicative usually of a mild infection. The monocytes extended to the edge of the nodule where they were seen in larger clusters. On the outer surface of the nodule were seen clusters of red blood cells. The ectoderm appeared thickened in some regions underlying the inoculum, due to a marked proliferation of ectodermal cells. Just underlying the area of marked ectodermal proliferation were seen fibroblasts and groups of proliferating ectodermal cells, interspersed among which were red blood cells and leukocytes. Beneath this area in the mesoderm were small groups of leukocytes and mesenchymal cells. In areas of the mesoderm, but not directly associated with the inoculum, were large groups of ectodermal cells showing pearl formation, indicating hyperkeratinization, and infiltrated with

leukocytes and some red blood cells. The entoderm appeared unaltered.

The inoculum itself exhibited a pink-staining, translucent, granular exudate with the fungi staining only with eosin in the center and base of the growth. The exudate merged with the ectoderm. The fungi at the periphery, however, showed a well developed growth which stained with methylene blue and had the characteristic appearance of the spherical cells and filaments seen in scrapings of a human lesion (Fig. 1).

B. *Pityrosporum ovale* (Bizzozero) Castellani and Chalmers, 1913.

Eggs were incubated 13 days and observed 5 days after inoculation. Macroscopically, the membrane showed some discrete, fine opacities over its surface. The embryos were alive.

Microscopically, groups of ovoid to spherical, budding, yeast-like organisms were seen on the ectoderm (Fig. 2). The ectodermal layer showed some proliferation and thickening, with vacuolated cells with a few inclusions of the sort that have been termed pseudo-inclusions by some authors. In places the organisms had invaded the ectoderm. The mesoderm was hyperplastic, and infiltrated by ectodermal cells, many monocytes, red blood cells and a few leukocytes. There was some endothelial hyperplasia. The entoderm was very slightly changed, if at all.

II. Dermatomycoses (epidermic and dermic lesions)

A. *Trichophyton gypseum* Bodin, 1902.

Eggs were incubated 13 days and observed 5 and 8 days after inoculation. Macroscopically, the growth on the membrane appeared as confluent colonies of aerial mycelium (Fig. 3). The membrane showed a grayish infiltrate, appeared greatly thickened immediately surrounding the macroscopic growth, and was white in color. The embryos were alive.

Microscopically, the growth on the chorio-allantois and the reaction of the tissue simulated very closely a "traumatic ulcer." The ectoderm had lost its identity in the central area underlying the inoculum, having become infiltrated with a layer of monocytes which lay between the fungous growth and the membrane. Many monocytes were interspersed among the fungous elements, par-

ticularly at the periphery of the inoculum adjoining the membrane. In addition, a large number of red blood cells were seen at the periphery and somewhat on the surface of the growth. The ectoderm was markedly thickened and hyperplastic in areas, but was largely replaced by inflammatory granulation tissue arising from the mesoderm. A layer of fibroblasts lay just beneath the replaced area of ectoderm. The mesoderm was markedly edematous, showing numerous islets of ectodermal cells, a huge number of fibroblasts, leukocytes and monocytes with a perivascular infiltration, endothelial cell hyperplasia and increased number of distended capillaries. The marked edema in the area underlying the inoculum had resulted in a greatly thickened membrane. The part of the entoderm lying directly under the center of the inoculum was also very hyperplastic with elongated papillae merging into the normal portion of the layer. The whole reaction varied in intensity in relation to the center of the inoculum, being very marked in the center of the membrane and becoming less toward the edge of the fungous growth.

The fungous elements adjoining the ectoderm had the morphology and characteristics of those seen in culture. At the periphery of the growth, however, the fungus appeared as fine filaments and small, spherical spores without the large round cells seen in culture (Fig. 4).

B. *Epidermophyton inguinale* Sabouraud, 1910.

Eggs were incubated 13 days and observed 8 days after inoculation. Macroscopically, the membrane was thickened by a marked confluent growth of the inoculum with aerial mycelium. Surrounding the inoculum there was a grayish, thickened opaque area. The embryos were dead.

Microscopically, the lesion simulated closely a "traumatic ulcer" showing loss of continuity of the ectoderm and replacement with inflammatory tissue arising from the mesoderm. The ectoderm at the margins of the inoculum was greatly thickened, becoming normal beyond the zone of mesodermal edema which underlay the fungous growth. Necrosis was apparent in the area of the replaced ectoderm. Here there were leukocytes in stages of degeneration, fibroblasts, and a marked increase in capillaries packed with red blood cells. Immediately above this area numer-

ous monocytes formed a layer which was interspersed with the fungous elements. The mesoderm showed marked edema, cellular proliferation and migration of cells with many fibroblasts and ectodermal cells in islets or whorls in active proliferation. There were also groups of leukocytes, dilated capillaries, red blood cells, monocytes and occasionally eosinophil-like cells. The fibroblasts were more noticeable just under the inoculum. However, upon examination of the mesoderm on either side of the inoculum, leukocytes were observed in large numbers, decreasing as the normal area of the membrane was approached.

The entoderm, associated with the edematous area of the mesoderm, exhibited an increased proliferation which diminished in the direction of the nonaffected area of the mesoderm.

The organism, *E. inguinale*, as it appeared on the membrane, consisted of spherical cells which took only the eosin. Scattered among them were numerous monocytes, leukocytes and red blood cells. Going outward, toward the periphery of the inoculum, there were seen fewer spherical cells, but filaments were present which took the methylene blue stain in addition to the eosin. At the periphery of the inoculum, filaments and fuseaux were encountered chiefly, with some small round cells. But in sections of the ectoderm, where the organism was found in small collections, *i.e.*, in the area surrounding the inoculum, the fungus had invaded the ectoderm, reverted to its parasitic morphology as seen in human lesions and caused a thickening of the ectodermal layer (Figs. 5 and 6). The ectodermal cells had lost their normal morphology and had become degenerated and somewhat cornified, with a subsequent desquamation. In short, this reaction simulated a typical epidermophytosis.

C. *Achorion Schoenleini* (Lebert) Remak, 1845.

Eggs were incubated 12 days and observed 5 days after inoculation. Macroscopically, the membrane exhibited a growth consisting of aerial mycelium with a hazy grayish infiltration in the membrane, rather similar to that of *T. gypseum*. The live embryos showed large gas bubbles in the chorio-allantois, which was a characteristic found in all eggs inoculated with *A. Schoenleini*.

Microscopically, the ectoderm underlying the fungous growth was discontinuous, with marked proliferation of the ectodermal

cells present and an edematous mesodermal infiltration associated with them (Fig. 8). The base of the inoculum, closely applied to the membrane, was infiltrated with monocytes, leukocytes and red blood cells. The mesoderm showed numerous fibroblasts and leukocytes, some monocytes, many capillaries with red blood cells, proliferating ectodermal cells and eosinophil-like cells. These various cells were more numerous in the region underlying the fungous growth and became fewer in the adjacent normal area. The entoderm beneath this infiltrate was hyperplastic.

The fungous growth was infiltrated by leukocytes and red blood cells. The fungus showed the characteristics of the organism in culture at the base of the growth, but took on the parasitic characteristic of short filaments at the periphery of the growth and in isolated regions where a small amount of the fungus was associated with the membrane (Fig. 7).

D. *Microsporum canis* Bodin, 1904.

Eggs were incubated 10 days and observed 5 days after inoculation. Macroscopically, the membrane showed a thick growth at the inoculum which was raised at the periphery with a grayish infiltrate in the membrane. The embryos were dead and the yolk was murky. The membranes were thickened.

Microscopically, the membrane exhibited a marked reaction to the presence of *M. canis*, the whole appearing very much as a "traumatic ulcer" as shown with several other fungi. The fungous growth extended along the surface of the broken ectoderm, which was replaced by cells of the inflammatory granulation tissue which had migrated from the mesoderm. The ectoderm, in the intact areas and particularly at the edges of the fungous growth, was much thickened and showed proliferating islets or groups of ectodermal cells extending into the edematous region at the base of the translucent, granular, fungus-invaded material adjoining the inflammatory area (Fig. 9). The base of the fungous growth was heavily infiltrated with monocytes, red blood cells, leukocytes and some degenerated cells. These cells were also interspersed among the clusters or islets of ectodermal cells of the discontinuous ectoderm.

The region of the mesoderm just below the necrotic ectodermal layer showed compact massing of fibroblasts and leukocytes,

which seemed to be migrating toward the center of the overlying fungous growth. The mesoderm itself showed a marked cellular accumulation in this region and a generalized edema in the area of the fungous growth. The cellular infiltration consisted of large numbers of fibroblasts, leukocytes and red blood cells. There were many capillaries, some monocytes, and actively proliferating whorls of ectodermal cells surrounded in most cases by leukocytes. The fibroblasts were most numerous near the ectoderm, whereas large groups of leukocytes were prevalent deeper in the mesoderm and closer to the entoderm. The entoderm was somewhat thickened, but proliferation was not marked.

The fungous growth was seen in the translucent, granular material adjoining the ectoderm as filaments and spores with some monocytes scattered throughout. At the periphery of the growth the fungus consisted of short filaments and numerous, somewhat encapsulated spores. Eggs inoculated with a freshly isolated culture exhibited characteristic filaments and fuseaux typical of *M. canis* in culture.

III. Cutaneous, subcutaneous, mucous membrane and internal organ involvement (localized cutaneous lesions, granulomata, deep-seated ulcerative lesions, mucous membrane plaques, lymph stream invasion with dermic and subsequent epidermic involvement, and visceral or generalized diseases)

A. *Monilia albicans* Zopf, 1890.

This organism is likewise referred to as *Syringospora albicans* (Robin) Dodge, 1935.

Eggs were incubated 12 days and observed 6 days after inoculation. Macroscopically, the membrane showed diffuse to confluent grayish, opaque plaques, thicker than the apparently unaffected areas. The embryos were dead.

Microscopically, the membrane showed hyperactivity indicative of a marked response to the foreign organism (Fig. 11). The ectoderm was thickened, particularly at the margins of the lesion. Here there was active proliferation of the ectodermal cells with an infiltration of leukocytes, monocytes and red blood cells, some in various stages of degeneration. The ectoderm was not continuous in the area associated with the fungous growth, although its

character was maintained elsewhere. In places the ectodermal cells were flattened and elongated with evidence of monocytic invasion overlying this layer and invading the fungous growth. In other areas the ectoderm seemed to cornify in layers, on the surface, in the act of desquamation, while the underlying ectodermal cells were actively proliferating in scattered whorls. A fine, granular exudate was present on the desquamating ectoderm and in numerous areas the budding cells had invaded the greatly thickened, cornified ectoderm.

The reaction of the mesoderm to *M. albicans* was remarkable. In the areas of greatest activity, *i.e.*, under the marginal ectodermal thickening, there were whorls of actively proliferating ectodermal cells extending into the mesoderm, some appearing as elongated projections. Fibroblasts occurred just beneath the ectoderm, with numerous accumulations of leukocytes in whorls, often showing degeneration. There were many thrombosed capillaries. Most interesting, however, was the formation of many pearls of apparently ectodermal origin indicative of hyperkeratinization (Figs. 12 and 13). This process was analogous in all respects to that in infection of human epithelium with the same organism. The whole picture was one of edema with increased cellular activity and resultant thickening of the membrane in the involved area. The entoderm was not significantly altered except for slight thickening in the areas of mesodermal and ectodermal response, with some accumulation of leukocytes on its mesodermal surface.

This fungous growth is of interest also since the inoculum adjoining the ectoderm, except at the surface of the growth, induced the formation of an exudate, probably arising from the chorioallantois, which was pink staining and within which could be seen filaments of the organism, red blood cells, monocytes and a few budding, yeastlike cells. At the periphery of the growth, however, the organisms were almost exclusively budding, and scattered among them were monocytes and red blood cells and the exudative, granular material (Fig. 10). The fungus here was of the type seen in human lesions.

B. *Geotrichum versiforme* Moore, 1934.

Eggs were incubated 12 days and observed 5 days after inoculation. The membrane macroscopically showed grayish yellow,

thickened plaques, some confluent, others diffuse, producing a thickened membrane. The embryos were dead.

Microscopically, there was a marked edematous reaction throughout the fungus-invaded areas and in the membrane (Fig. 15). The reaction simulated an ulcer to a certain extent, with the ectoderm broken in parts and replaced with inflammatory migrating cells from the mesoderm and from the proliferating ectoderm. The ectoderm was greatly thickened in areas with degenerated cells, red blood cells, monocytes and leukocytes. The marginal regions of the fungous growth on the ectoderm showed proliferation and thickening of the ectoderm. The mesoderm was edematous with many thrombosed capillaries, whorls or islets of proliferating ectodermal cells and several ectodermal pearls. Just beneath the ectoderm were fibroblasts and leukocytes with a number of small thrombosed capillaries. Scattered throughout the membrane were red blood cells and numerous basophilic leukocytes occurring in clusters or singly. The entoderm was thickened and proliferated below the regions of active ectodermal reaction, with many fibroblasts and capillaries in the adjacent mesoderm.

The fungous growth on the ectoderm was infiltrated by many inflammatory cells, such as leukocytes in various stages of degeneration, red blood cells, monocytes and some ectodermal cells. In several areas the fungus, *G. versiforme*, was composed of filaments and rectangular to ovoid cells. When associated with the membrane or the inflammatory cells of the membrane, the cells were the type seen in human tissue, somewhat rectangular to spherical or ovoid (Fig. 14).

C. *Zymonema dematitidis* (Gilchrist and Stokes) Dodge, 1935.

Eggs were incubated 12 days and observed 5 and 10 days after inoculation. Macroscopically, eggs inoculated for 5 days showed a mat of aerial mycelium on the membrane growing as a colony. The embryos were alive. Inoculated eggs observed after 10 days showed rather dry membranes, fairly thin and somewhat broken up. Distributed over the intact membrane were nodules which were yellow in color. The embryos were dead.

Microscopically, the younger membranes (5 days) showed an invasion by the fungus with a consequent loss in character of the

various layers. There was a marked monocytic infiltration in the mesoderm with some whorls of ectodermal proliferation and some leukocytes. The fungus had the characteristics of the usual cultural growth, but showed, on the fifth day, the beginning of spherical cell formation (Fig. 16).

Membranes examined 10 days after inoculation were extremely thin and broken, showing ectodermal proliferations and cornification, with an almost complete loss of mesoderm. Scattered over the membrane were small nodules which on microscopic examination were found to be composed of several zones. There was some ectodermal thickening at the margins of the fungus growth. The region of the nodule adherent to the thin, cornified membrane showed a layer of monocytes. The central portion of the nodules was seen to consist of budding, thick-walled, yeastlike cells, comprising the parasitic type of *Zymonema dermatitidis* (Fig. 17). The organism in 10 days thus showed a reversion to a parasitic rôle.

D. *Cryptococcus hominis* (*Cryptococcus histolyticus*) (Busse) Vuillemin, 1901.

Eggs were incubated 11 days and observed 5 days after inoculation. Macroscopically, there was a grayish, moist mat over the membrane with resultant thickening. The embryos were alive.

Microscopically, the chorio-allantois showed little response to the fungus except for a thickening of the ectoderm where it came in contact with the organism and a leukocytic infiltrate in the mesoderm which was not marked. The entoderm was not affected. The fungus-affected area exhibited a marked infiltration of monocytes with some red blood cells. The organism, *C. hominis* or *C. histolyticus,* was seen as a budding, yeastlike, mucoid-encapsulated cell, characteristic of the parasitic stage of the organism (Fig. 18).

E. *Coccidioides immitis* Stiles, 1896.

Eggs were incubated 13 days and observed 7 days after inoculation. Macroscopically, the membrane showed grayish yellow, thickened plaques which were somewhat confluent. The embryos were alive.

Microscopically, the chorio-allantois showed little response to

the organism except for a thickening of the ectoderm where it came in contact with the fungous growth. In some areas the ectoderm showed necrosis with an invasion of leukocytes which had undergone degeneration. The mesoderm in some areas showed focal invasion with leukocytes as well as scattered leukocytes throughout. The entoderm appeared unaltered. The surface of the membrane, however, showed a granular exudate in areas both on the ectoderm and entoderm, with numerous red blood cells. On the ectodermal surface, in addition, there was a more or less translucent, granular exudate which appeared in layers or striations, staining intensely with eosin (Figs. 19 and 20). This material covered most of the ectodermal surface and showed many red blood cells. Within this material, *C. immitis* could be seen in various stages of development, with the formation of the characteristic endosporulating cells of the parasitic stage of the fungus. The inoculated filaments were first converted into arthrospores, and then into spherical cells which enlarged and developed endospores. In 7 days on the chick membrane the organism had shown a reversion to its parasitic rôle which in animals usually takes a much longer time.

F. *Sporotrichum Schencki* Matruchot, 1910.

Eggs were incubated 13 days and observed 10 days after inoculation. Macroscopically, the membrane showed grayish, somewhat yellowish, thickened areas on its surface, somewhat raised in parts with a hazy infiltrate in the surrounding area. Embryos were dead.

Microscopically, the picture was somewhat confusing. The membrane showed edema with a large number of red blood cells, leukocytes, migrating cells, fibroblasts and monocytes, many showing degeneration. The ectoderm in many areas was necrotic and presented an inflammatory infiltration. In other areas, just underlying the heaviest growth of the inoculum, the ectoderm showed marked proliferation at its base and cornification. Just underlying the cornified ectoderm and displacing part of the mesoderm was a granular infiltration with groups of the cigar-shaped, parasitic cells of *S. Schencki* scattered throughout. Adjoining the exudate and extending into the mesoderm were degenerated leukocytes, some fibroblasts, red blood cells, and some

monocytes. The mesoderm showed leukocytes scattered throughout, many of them degenerated, and some monocytes and fibroblasts. The entoderm was somewhat thickened, necrotic in areas and somewhat broken through with a granular exudate on some areas of its inner surface. On its outer surface was present the same granular exudate, although more abundant, with numerous leukocytes, many degenerated.

The fungous inoculum was very interesting, having three zones (Fig. 21). At the base of the inoculum, adjoining the cornified ectoderm, were monocytes which were found in greater number at the margin of the growth. They were seen in groups dispersed through the fungous growth. The base of the inoculum was made up almost exclusively of round spores of the fungus. The center of the inoculum showed a translucent, granular substance which stained with eosin. Dispersed through this area were the fine, branching filaments of the organism, with some conidia. This region was flanked by spores and monocytes. The periphery of the inoculum showed clusters of the cigar-shaped cells of *S. Schencki,* the parasitic cells of the fungus (Fig. 22).

G. *Actinomyces bicolor* Trolldenier, 1903.

Eggs were incubated 11 days and examined 5 and 8 days after inoculation. Macroscopically, the membranes may show a single inoculation with a densely opaque area surrounding the implant and a lighter opaque area extending from the denser region (an ectodermal involvement) (Fig. 23), or they may show small, diffuse nodules on a densely opaque area with *Actinomyces bovis* (Fig. 24). The embryos were alive.

Microscopically, the chorio-allantois showed a marked reaction to the fungus, with edema and thickening of the membrane. The ectoderm associated with the growth was greatly thickened and proliferative, with a marked infiltration of monocytes, leukocytes and red blood cells, some of which had degenerated. The ectoderm was irregular and broken in areas where the infiltration was particularly heavy. The fungous growth on this layer was irregular in outline and was made up of fine, branching filaments with spore formation, many of the filaments having invaded the ectoderm itself (Fig. 25). The growth was also infiltrated with monocytes, leukocytes and some red blood cells. The mesoderm showed inflammation and edema with an invasion of many whorls

of proliferative ectodermal cells. These in turn were surrounded by numerous leukocytes. The leukocytes were seen in large numbers throughout this layer, as well as fibroblasts and thrombosed capillaries. Except for a few sections that were somewhat thickened, the entoderm did not seem to be affected.

H. *Monosporium apiospermum* Saccardo, 1911.

Eggs were incubated 13 days and observed 5 days after inoculation. Macroscopically, the membrane appeared considerably thickened in the affected areas, showing thick, grayish, opaque infiltrations either confluent or diffuse (Fig. 26). The embryos were alive.

Microscopically, there was very little differentiation of any of the layers of the membrane, since the fungus had grown through and involved the whole chorio-allantois (Fig. 28). The result was a thickened growth which was differentiated only according to the growth of the fungus. Dispersed through the affected membrane, however, in regions which the organism had not invaded completely, were seen groups of monocytes, some leukocytes and red blood cells. In other areas, within the membrane, were seen groups of single spores of *M. apiospermum* (Fig. 27).

The growth appeared in various layers. The peripheral growth stained with methylene blue and was seen as a loose network of fine filaments and small spores. In other areas there was a growth outward, onto the surface of the membrane, showing clusters of larger spores, more typical of *M. apiospermum* in culture. The second layer consisted of closely interwoven hyphae which stained only with eosin. The third area consisted of loosely intertwined and growing mycelium with clusters of spores comparable in size to those seen on the surface of the membrane. The growth of the fungus and the resultant change in the membrane indicated, in a measure, the type of pathology provoked in humans—that of a granuloma.

I. *Phialophora verrucosa* Thaxter, 1915.

Eggs were incubated 12 days and observed 5 and 8 days after inoculation. Macroscopically, the membrane showed yellowish to light brown plaques with a hazy gray infiltration in the surrounding area. The embryos were alive.

Microscopically, the membrane had developed a severe reac-

tion to the presence of the fungus, with edema, inflammation and resultant thickening and proliferation of the various layers. The ectoderm showed marked hyperplasia and proliferation with an invasion by the fungus, *P. verrucosa* (Fig. 29). There was a heavy infiltration of monocytes in the region where the fungus invades the ectoderm. The invaded ectoderm showed whorls of proliferating ectodermal cells which extended into the edematous mesoderm. The region underlying the involved ectoderm had marked ectodermal proliferation and migrating fibroblasts, and scattered among these were leukocytes and thrombosed capillaries. The mesoderm showed an inflammatory process with numerous leukocytes, whorls of ectodermal cells and capillaries which extended down to the entoderm. The entoderm was very little affected in spite of the reaction in the other two layers.

The fungous growth on the ectoderm consisted of an outer growth of loose filaments and round cells. The inner area had a somewhat translucent, granular material which stained with eosin and within which were seen the hyphae, round cells and some phialides of *P. verrucosa*.

Summary and Conclusions

A number of fungi representative of the causative agents of various types of lesions which develop in man were successfully inoculated in the chorio-allantoic membrane of the developing chick to determine the effect on the fungi and the reactivity of the chorio-allantois to the parasites. Fertilized eggs that were incubated 10 to 14 days were used in this study. Inoculations were made directly on the chorio-allantoic membrane. Macroscopically, the lesions manifested themselves as thick or thin, white, grayish or grayish yellow to light brown, confluent or discrete plaques on the membrane, depending on the type of organism used.

Microscopically, the membrane reacted in the form of nodules, ulcers, superficial growths and hyperplastic lesions showing varying degrees of proliferation. There was increased activity in most cases as evidenced by intense infiltration of ectodermal cells, red blood cells, fibroblasts, leukocytes and monocytes, with inflammatory changes in the mesoderm and ectoderm, and in some cases in the entoderm, and marked edema at the sites of fungous growth. This resulted in a thickening of the membrane which was in-

creased also by the invasiveness, in some cases, of the fungous elements.

The fungi grew luxuriantly on the chorio-allantois, were easily demonstrated with methylene blue and eosin, and in most cases showed a reversion to their parasitic morphology in from 5 to 11 days. Strangely enough, with yeastlike organisms this reversion may take place throughout the entire growth or, as is the case with some of the filamentous forms, at the periphery of the fungous inoculum, in the ectodermal layer or at the surface of the ectoderm where the amount of mycelium is relatively small.

By this method it has been possible to develop lesions, some of which have hitherto required human subjects. The value of the chorio-allantois for this purpose may be emphasized also because the cost is much less than if experimental laboratory animals are used and the time necessary for the development of diagnostic features frequently is much shorter. The gratifying results which have been obtained indicate great possibilities in investigative work with fungi and thus warrant the continued use of this method in mycology.

REFERENCES

1. Goodpasture, E. W. Some uses of the chick embryo for the study of infection and immunity. *Am. J. Hyg.*, 1938, **28**, 111–129.

2. Wolff, Max, and Israel, James. Ueber Reincultur des Actinomyces und seine Uebertragbarkeit auf Thiere. *Virchows Arch. f. path. Anat.*, 1891, **126**, 11–59.

3. Levaditi, C. La spirillose des embryons de poulet dans ses rapports avec la tréponémose héréditaire de l'homme. *Ann. Inst. Pasteur*, 1906, **20**, 924–938.

4. Rous, Peyton, and Murphy, James B. Tumor implantations in the developing embryo. Experiments with a transmissible sarcoma of the fowl. *J. A. M. A.*, 1911, **56**, 741–742.

5. Clark, Eliot R. Technique of operating on chick embryos. *Science*, 1920, **51**, 371–373.

6. Woodruff, Alice Miles, and Goodpasture, Ernest W. The susceptibility of the chorio-allantoic membrane of chick embryos to infection with the fowl-pox virus. *Am. J. Path.*, 1931, **7**, 209–222.

7. Goodpasture, Ernest W., and Buddingh, G. John. The preparation of anti-smallpox vaccine by culture of the virus in the chorio-allantoic membrane of chick embryos, and its use in human immunization. *Am. J. Hyg.*, 1935, **21**, 319–360.

8. Burnet, F. M. The use of the developing egg in virus research. *Medical Research Council, Special Rep. Ser., No. 220*, His Majesty's Stationery Office, London, 1936.

9. Morrow, Grant; Syverton, Jerome T.; Stiles, William W., and Berry, George Packer. The growth of *Leptospira icterohemorrhagiae* on the chorio-allantoic membrane of the chick embryo. *Science*, 1938, **88**, 384–385.

10. Moore, Morris. The chorio-allantoic membrane of the developing chick as a medium for the cultivation and histopathologic study of pathogenic fungi. *Science*, 1939, **89**, 514–515.

DESCRIPTION OF PLATES

PLATE 23

FIG. 1. Parasitic type of *Malassezia furfur* in periphery of inoculum (5 days). × 350.

FIG. 2. Section through membrane showing *Pityrosporum ovale* on, and invading, the ectoderm (5 days). × 430.

FIG. 3. Chorio-allantoic membrane infected with *Trichophyton gypseum*. Egg incubated 10 days and observed 7 days after inoculation.

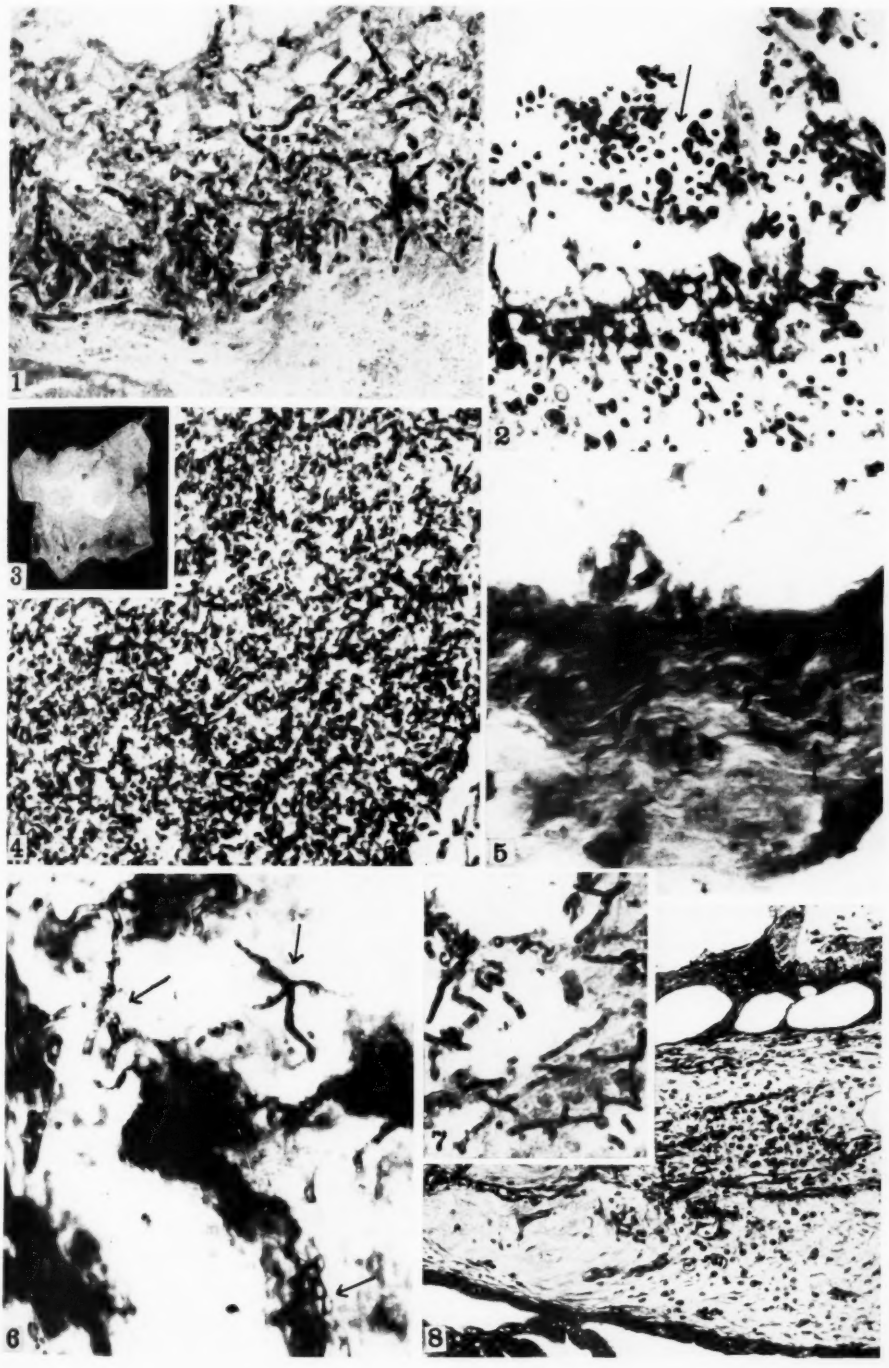
FIG. 4. Small spores and fine filaments in peripheral growth of *Trichophyton gypseum* (8 days). × 330.

FIG. 5. *Epidermophyton inguinale*. Cornified ectoderm with invading fungous filaments (8 days). × 330.

FIG. 6. Parasitic type of filaments of *Epidermophyton inguinale* as seen in cornified material on ectoderm (8 days). × 390.

FIG. 7. Parasitic filaments of *Achorion Schoenleini* in ectodermal detritus (5 days). × 430.

FIG. 8. Section through membrane inoculated with *Achorion Schoenleini*. Note gas bubbles in ectoderm (5 days). × 135.



Moore

Chorio-Allantoic Cultivation of Fungi

PLATE 24

FIG. 9. *Microsporum canis*. Fungous elements in translucent, granular exudate. Note marked ectodermal proliferation (5 days). × 95.

FIG. 10. Section through peripheral growth showing parasitic, budding cells of *Monilia albicans*, with monocytes and some leukocytes (6 days). × 800.

FIG. 11. *Monilia albicans*. Section through fungous growth and part of membrane. Note marked growth at periphery (6 days). × 80.

FIG. 12. Section through membrane inoculated with *Monilia albicans*. Note whorls of ectodermal cells and marked pearl formation in mesoderm (6 days). × 85.

FIG. 13. Ectodermal pearls in membranes infected with *Monilia albicans*, higher magnification (6 days). × 310.

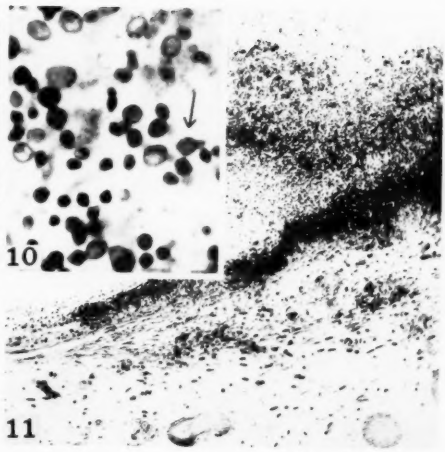
FIG. 14. Parasitic cells of *Geotrichum versiforme* associated with inflammatory cells (5 days). × 350.

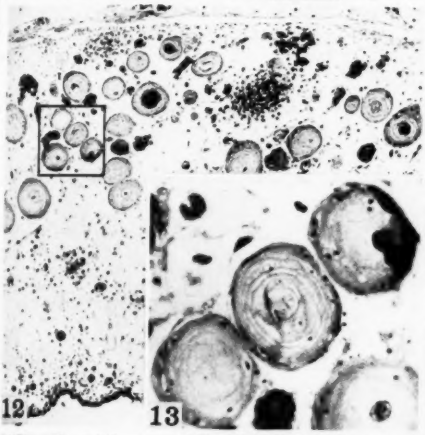
FIG. 15. *Geotrichum versiforme*. Section through fungous inoculum and portion of membrane. Note broken ectoderm and irregular fungous growth (5 days). × 80.

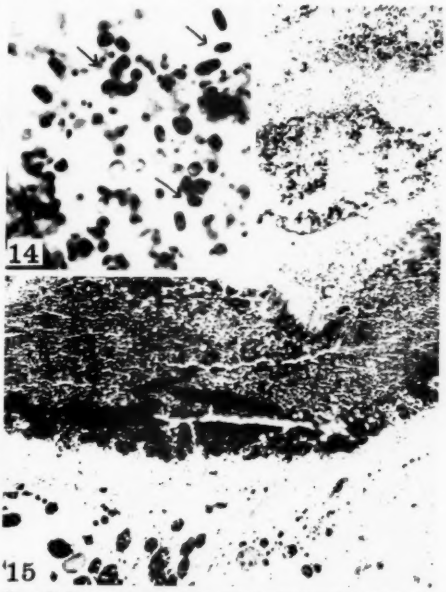
FIG. 16. Section through membrane invaded by *Zymonema dermatitidis*, showing the formation of spherical cells (5 days). × 440.

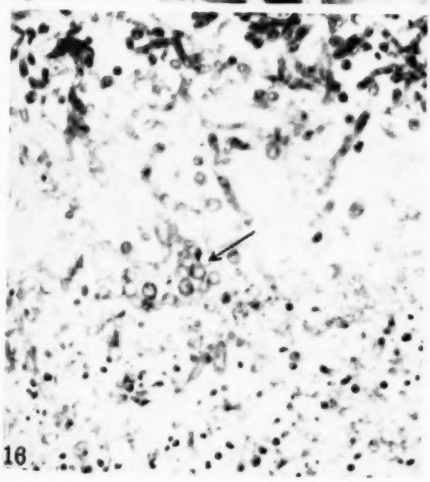
FIG. 17. Budding, thick-walled, yeastlike, parasitic cells of *Zymonema dermatitidis* in nodule formation on membrane (10 days). × 745.


9


10
11


12
13


14
15


16

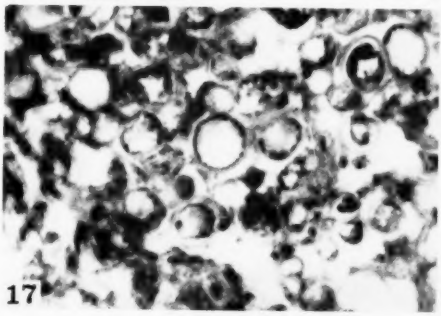

17

PLATE 25

FIG. 18. Mucoid-encapsulated, budding, parasitic cells of *Cryptococcus hominis* on membrane (5 days). × 605.

FIG. 19. *Coccidioides immitis*. Transformation of hyphal cells to endosporulating structures as seen in exudate (7 days). × 445.

FIG. 20. Endospore formation of *Coccidioides immitis*, partly hidden by overlying section, embedded in translucent, granular exudate (7 days). × 430.

FIG. 21. Section through inoculum and portion of membrane. Note translucent, granular exudate within which are seen the fine filaments of *Sporotrichum Schencki*. Note also reaction on ectoderm (10 days). × 85.

FIG. 22. Section of periphery of inoculum showing typical, cigar-shaped, parasitic cells of *Sporotrichum Schencki* occurring in clusters (10 days). × 880.

FIG. 23. Membrane infected with *Actinomyces bicolor*. Egg incubated 10 days and observed 7 days after inoculation.

FIG. 24. Membrane infected with *Actinomyces bovis*. Egg incubated 10 days and observed 7 days after inoculation.

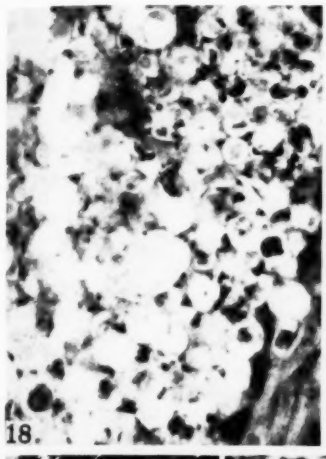
FIG. 25. Filaments and spores of *Actinomyces bicolor* in ectoderm (8 days). × 1080.

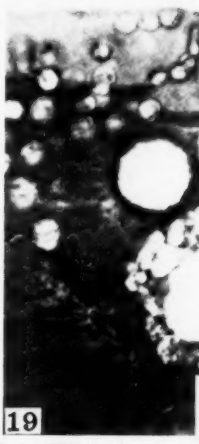
FIG. 26. Membrane infected with *Monosporium apiospermum*. Egg incubated 10 days and observed 7 days after inoculation.

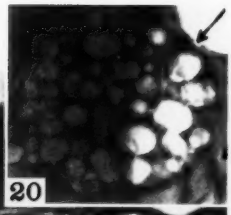
FIG. 27. Conidia of *Monosporium apiospermum* in the membrane (5 days). × 415.

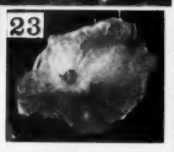
FIG. 28. Section through membrane showing zone formation and complete invasion by *Monosporium apiospermum* (5 days). × 80.

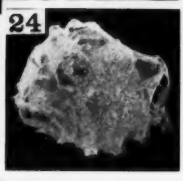
FIG. 29. Ectodermal proliferation as a result of presence of *Phialophora verrucosa* (8 days). × 305.

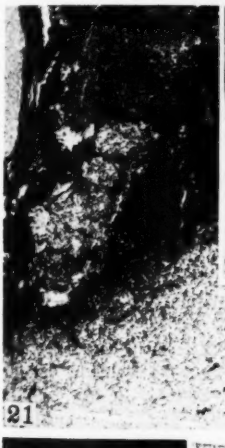

18.

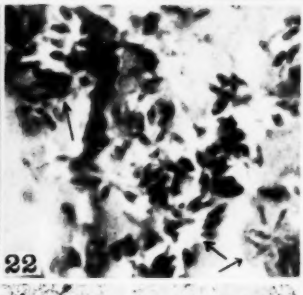

19


20


23

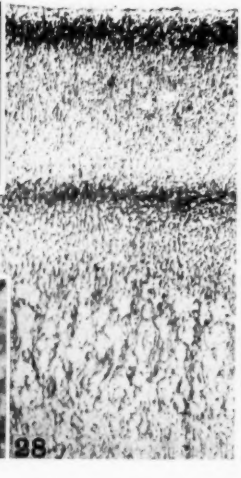

24

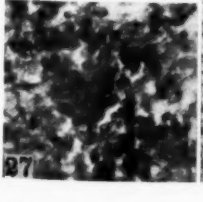

21

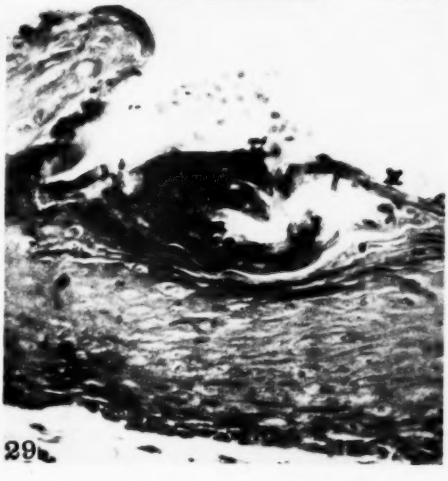

22


25


26


28


27


29

RHABDOMYOMATOSIS OF THE HEART IN A GUINEA PIG *

W. C. HUEPER, M.D.

(From the Warner Institute for Therapeutic Research, New York, N.Y.)

It is only in recent years that congenital rhabdomyomas of the heart have been reported, not only in the white race—48 cases; but also in other races—Negro, 1 case, and Japanese, 2 cases (Hueper;[1] Mitani;[2] Tamura[3]). The observation of rhabdomyomatosis of the heart in a guinea pig extends the occurrence of these rare blastomatoid formations of the cardiac muscle to a second species.

The present observation was the result of an incidental finding in a guinea pig used in an experimental investigation of the morphological action of digitalis glycosides upon the heart muscle. The animal died 4 days after the intramuscular injection of a digitalis preparation. Its weight was 245 gm. No information was available regarding its age. When examined *post mortem* the lungs were found to be congested and spotted with small dark red hemorrhagic areas. The left ventricle of the heart was firmly contracted, while the right ventricle was dilated. The heart measured 2 by 1 cm. On longitudinal sectioning it exhibited an irregular, indistinctly outlined, pale whitish red area measuring 0.5 by 0.75 cm. located in the apical portion of the left ventricular wall. The other organs were normal. The brain was not removed.

The lung, heart, liver, stomach, spleen, pancreas, suprarenal, kidney, testis and epididymis were examined histologically. Inasmuch as the pathological lesions noted were unrelated to the condition observed in the heart, it will suffice to list briefly the histological diagnoses made: purulent bronchitis, purulent pneumonia, marked congestion and edema of the lung; mild pericentral fatty infiltration of the liver; hemosiderosis of the spleen; moderate degeneration of the spermatogenic epithelium of the testis with accumulation of immature and partly degenerated spermatogenic cells within the epididymic ducts.

Sections from the heart were prepared from the wall of the left

* Presented at the Thirty-Third Annual Meeting of the American Association for Cancer Research, Pittsburgh, Pa., March 19, 1940.

Received for publication April 15, 1940.

ventricle, interventricular septum with left auricle and wall of right ventricle and auricle. The sections were stained with hematoxylin and eosin. Microscopical study showed that in addition to the large macroscopically visible focus in the wall of the left ventricle, there were numerous smaller areas of the same spongy tissue in other parts of the heart including the interventricular septum, right ventricular wall, right and left auricular walls and various papillary muscles. A subendocardial location of these foci was noted rather frequently, causing a slight to moderate local bulging of the wall into the ventricular cavities. The nodules composed of muscular tissue with large vacuoles were indistinctly outlined from the normal myocardial tissue and seemed to merge with it in many areas of their circumference. Normal myocardial muscle bundles and strands of vesicular, swollen, primitive, immature muscle cells were found interlocked in such regions.

The pathological tissue was composed of a spongy network in which more or less wavy, delicate fibrils surrounded huge, irregularly round, polygonal or oblong vacuoles, producing thereby the picture of a tissue consisting of an accumulation of partly collapsed and more or less empty bags. The majority of the vacuolar spaces were empty after having been subjected to the various procedures of fixation, dehydration and staining, but an appreciable number of them displayed some type of content. In most, a chromatic, elongated nucleus was present, apparently flattened against the wall of the vacuole. In other cells there was a cytoplasmic marginal rim containing a somewhat larger, irregularly round nucleus projecting into the vacuolar center (Fig. 1). Some cells contained a pinkish granular material, the granules showing sometimes an abortive striated arrangement. Similar granular or homogeneous cytoplasmic matter was seen to surround a centrally located round nucleus from which ribbon-like or fibrillar cytoplasmic processes extended to the cellular periphery ("spider cells"). Scattered throughout this primitive, embryonic myocardial tissue there were cells containing fragments of cross striated sarcous material (Fig. 2), while some of the large cells had a foamy cytoplasm with hyaline, blotchy inclusions, staining dark pink. Small groups of cells stained dirty bluish gray were observed in several places within the spongy areas, evidently representing calcified, degenerated primitive myocardial cells. Staining

for glycogen was negative, but the heart had been for 1 week in a solution of formaldehyde before dehydration was started.

Comment

Inasmuch as the morphology of the spongy tissue areas in this heart duplicates exactly that seen in rhabdomyomas of the human heart, there can be no doubt that this is a case of rhabdomyomatosis of the heart of a guinea pig. The evidence presented supports the conception that rhabdomyomatous formations of the heart are not true tumors, but congenital tissue malformations with blastomatoid characteristics.

In a recent communication on von Gierke's glycogen-storage disease Humphreys and Kato [4] raised the question whether several of the examples reported as local or diffuse rhabdomyomatosis of the heart might not represent a myocardial variety of von Gierke's disease (Pompe [5]). It may be pointed out in this connection that the glycogen contained in the primitive muscle cells of rhabdomyomas apparently is very much more soluble in the ordinary fixatives than the glycogen stored in the various organ cells (liver, heart, etc.) in glycogen-storage disease. This difference is brought out very strikingly by the fact that the demonstration of glycogen in the cells composing rhabdomyomas has been accomplished only very rarely, because the glycogen in this instance had been removed from these cells by fixation in aqueous media before the proper staining procedures were applied, while the glycogen present in the tissues of von Gierke's disease has been demonstrated by chemical and staining methods after having been in fixating fluids for weeks or months. This glycogen is not only markedly resistant to postmortem hydrolysis, but also very much less soluble in watery agents than ordinary glycogen, including that contained in rhabdomyoma cells. This histochemical criterion may help in the future, in addition to the demonstration of "spider cells" and various myofibrillar evolutionary manifestations in the primitive myocardial cells, in distinguishing between rhabdomyomatous lesions and myocardial tissue changes associated with von Gierke's glycogen-storage disease.

REFERENCES

1. Hueper, W. C. Rhabdomyomatosis of the heart in a Negro. *Arch. Path.*, 1935, **19**, 372–379.

2. Mitani, Shigeru. Das kongenitale multiple Rhabdomyom des Herzens. *Tr. Soc. path. jap.*, 1934, **24**, 589.

3. Tamura, O. Über das Rhabdomyom des Herzens. *Gann*, 1936, **30**, 391–392.

4. Humphreys, Eleanor M., and Kato, Katsuji. Glycogen-storage disease, thesaurismosis glycogenica (von Gierke). *Am. J. Path.*, 1934, **10**, 589–614.

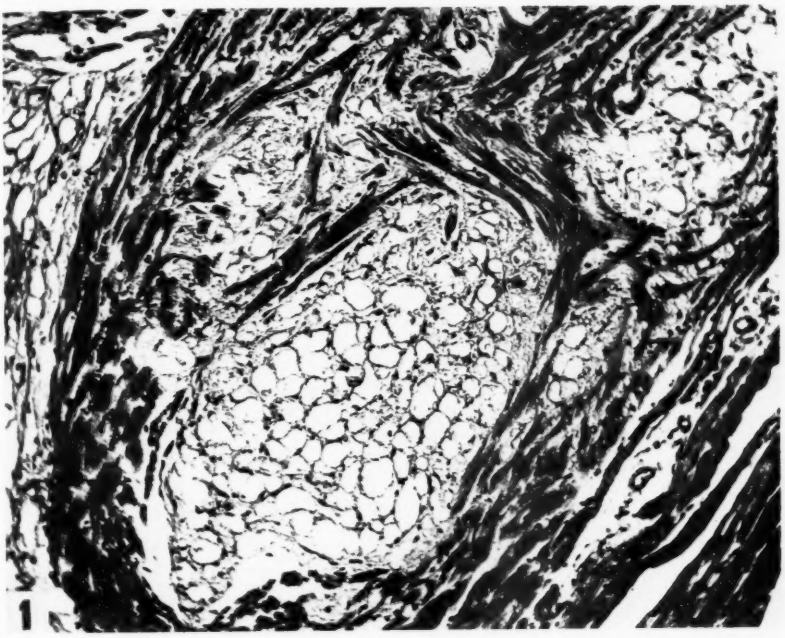
5. Pompe, J. C. Hypertrophie idiopathique du coeur. *Ann. d'anat. path.*, 1933, **10**, 23–35.

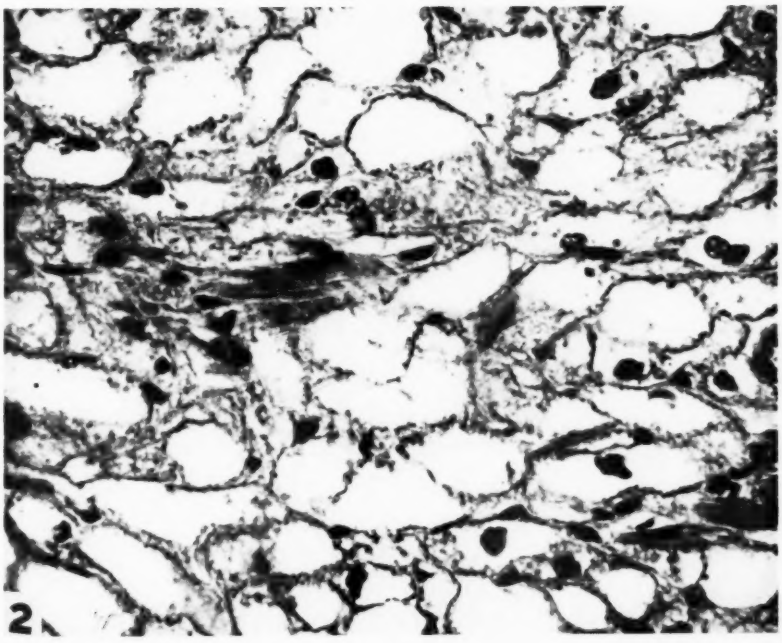
DESCRIPTION OF PLATE

Plate 26

Fig. 1. Rhabdomyomatous tissue embedded in normal myocardium, showing the typical large vacuolar structure. × 230.

Fig. 2. Rhabdomyomatous tissue with primitive myocardial cells containing fragments of cross striated sarcous material. × 625.





Hueper Rhabdomyomatosis of Heart in Guinea Pig

CONGENITAL NODULAR GLYCOGENIC DEGENERATION OF THE MYOCARDIUM *

RICHARD E. OLSEN, M.D., AND ROBERT J. COOPER, M.D.

(From the Pathological Laboratory, St. Joseph Mercy Hospital, Pontiac, Mich.)

Over fifty examples of so-called rhabdomyomatosis or congenital rhabdomyoma of the heart have appeared in medical literature, beginning with that described by von Recklinghausen [1] in 1862. The most recent summary is that of Labate [2] in 1939, which includes a bibliography and table of the significant findings. We present an additional case.

REPORT OF CASE

Clinical History. A white male infant weighing 8 pounds, 1 ounce was born uneventfully on June 20, 1940. It was the third sibling, with the first two living and well. During the first 10 days of life there was a mild diarrhea. This did not recur.

On July 28 the baby was readmitted to the hospital because of persistent vomiting after each feeding. He was dehydrated and his weight had dropped to 6 pounds. In spite of the administration of fluids and stimulants the infant died on July 29, the 40th day of life. The temperature rose to 109.4° F. before death.

POSTMORTEM EXAMINATION

An autopsy was performed 2 hours after death. The body was that of an emaciated, dehydrated white male infant weighing 3,100 gm. and measuring 54 cm. Externally there were no developmental disturbances.

The heart was normal in size but showed multiple nodules throughout the myocardium in both ventricles and auricles. These varied in size from those just visible to the naked eye up to 2 cm. in diameter. The larger nodules were found only in the ventricles. Some of the papillary muscles were involved. On cut surface the nodules were lighter in color than the normal muscle and were firm to the touch. This was the only cardiac disturbance found. The great vessels were normal.

The right lung had four lobes. The lower lobes of both lungs were atelectatic, probably due to pressure from the dilated

*Received for publication September 30, 1940.

stomach. The wall of the entire stomach was thickened and there was a heavy muscle band at the pylorus such as occurs with hypertrophic pyloric stenosis.

The kidneys were congested and a small abscess was noted at the lower pole on the left side. No developmental disturbance was present.

Unfortunately the brain was not examined. The spinal cord showed congestion.

Microscopic Examination

The nodules in the heart consisted of groups of hypertrophied muscle fibers with large intracellular vacuoles. The nuclei were not destroyed but were usually found near the margins of the cells and were distorted in shape. Most of the nodules were definitely demarcated but occasionally the outer zone was less involved and approached the normal myocardium in appearance. The interstitial tissue was not increased and there was no inflammatory reaction.

Upon removal the heart was first placed in an aqueous solution of formaldehyde (4 per cent). After 2 hours a portion of the heart was transferred to absolute alcohol. Sections of the alcohol-fixed material were stained for glycogen by Best's method. Glycogen was present in the vacuoles in the large nodules but was absent in the minute nodules. It is possible that the glycogen dissolved out of the small nodules while in the formaldehyde, but the fat stains did not suggest this. Frozen sections of the formaldehyde-fixed material were stained with Sudan III. Fine droplets of fat appeared throughout the small nodules and at the periphery of the large ones. No large fat droplets were found. The fat was present in the involved muscle fibers but not in the large glycogen vacuoles.

There was hyperplasia of the muscle fibers in the stomach wall with a slight patchy hypertrophy of the individual muscle fibers. Sections of the stomach wall were stained for fat and glycogen with negative results.

The kidneys showed an acute pyogenic process with one larger abscess as previously noted and multiple minute abscesses. There was no evidence of maldevelopment.

The spinal cord was congested and the anterior horn cells were undergoing an acute degeneration.

Summary. Dilatation of the stomach with generalized hypertrophy of the wall and pyloric stenosis. Acute purulent nephritis with gross and microscopic abscess formation. Multiple nodules in the auricular and ventricular myocardium containing glycogen and showing fatty degeneration (rhabdomyomatosis). Four-lobed right lung. Atelectasis of the lower lobes of both lungs. Acute degeneration of the anterior horn cells of the spinal cord. Marked emaciation and dehydration.

Discussion

The findings in this case support the conclusion of Steinbiss [3] that the myocardial nodules are a developmental disturbance with secondary degenerative changes. They are frequently associated with other anomalies, especially in the brain and kidney. Steinbiss concluded that the lesions are not neoplastic, and represent a local phase of a generalized condition rather than the mechanical type of malformation represented by tissue arrests. The individual lesions appear to be progressive and Steinbiss stated that they may, after degeneration, go on to scar tissue formation. There is no positive evidence that new lesions develop. The distribution of fat and glycogen in this case coincides with the description by Steinbiss but disagrees with that of Hueper [4] who found no fat in the frozen sections of the heart in his case.

The cardiac lesions in this and many of the other cases in the literature are not directly responsible for death. Not more than one patient in ten reaches the age of puberty and 50 per cent die in the first year of life.

Almost without exception there is no mention made of the auricles in the recorded cases. In this infant both auricles showed nodules, most of which were of minute size. The uniformly small size of the auricular nodules supports the theory that there is no hyperplasia of muscle fibers but only hypertrophy of individual groups.

Because of poor terminology this condition has been confused with the rhabdomyoma, which is a true neoplasm. For this reason it seems desirable to alter the terminology from congenital

rhabdomyoma or rhabdomyomatosis to a more suitable and distinctive term. *Congenital nodular glycogenic degeneration* of the myocardium agrees with the objective findings of the disease without suggesting a neoplastic origin or implying an exact knowledge of the etiology.

Summary

A white male infant, dying on the 40th day of life, showed multiple nodules throughout the ventricular and auricular myocardium. The nodules consisted of hypertrophied muscle bundles with fatty degeneration and glycogen deposits in large vacuoles. Other anomalies present were a four-lobed right lung and hypertrophy of the entire wall of the stomach with pyloric stenosis. This condition has been erroneously termed congenital rhabdomyoma or rhabdomyomatosis. We suggest, as a more suitable name, *congenital nodular glycogenic degeneration* of the myocardium.

REFERENCES

1. von Recklinghausen. Ein Herz von einem Neugebornen, welches . . . Tumoren (Myomen) trug. *Monatsschr. f. Geburtskunde*, 1862, **20**, 1–2.
2. Labate, John S. Congenital rhabdomyoma of the heart. Report of a case. *Am. J. Path.*, 1939, **15**, 137–150.
3. Steinbiss, W. Zur Kenntnis der Rhabdomyome des Herzens und ihrer Beziehungen zur tuberösen Gehirnsklerose. *Virchows Arch. f. path. Anat.*, 1923, **243**, 22–38.
4. Hueper, W. C. Rhabdomyomatosis of the heart in a Negro. *Arch. Path.*, 1935, **19**, 372–379.

DESCRIPTION OF PLATE

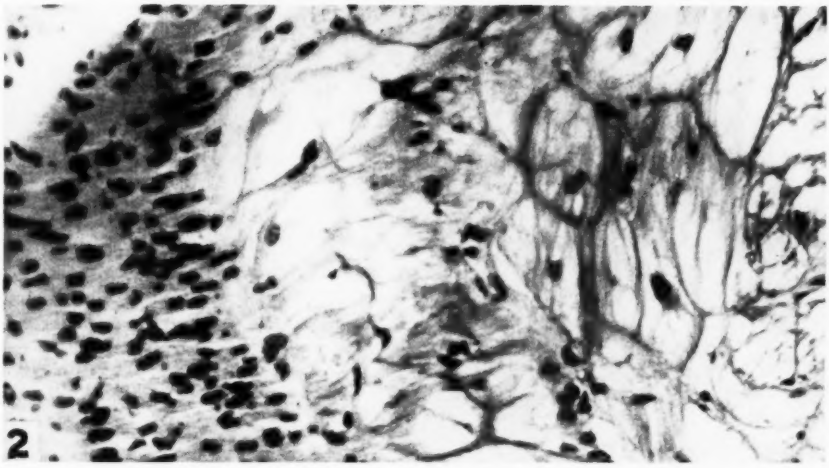
Plate 27

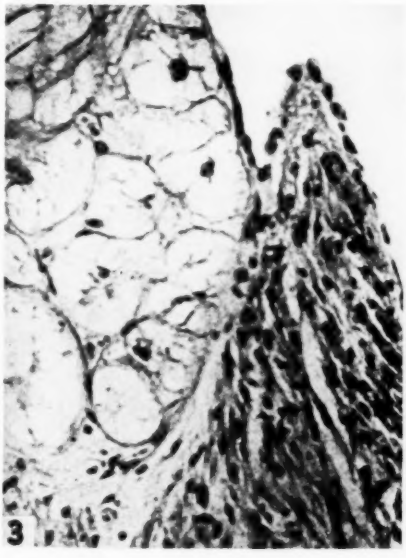
Fig. 1. Section through the wall of the left ventricle. × 3.5.

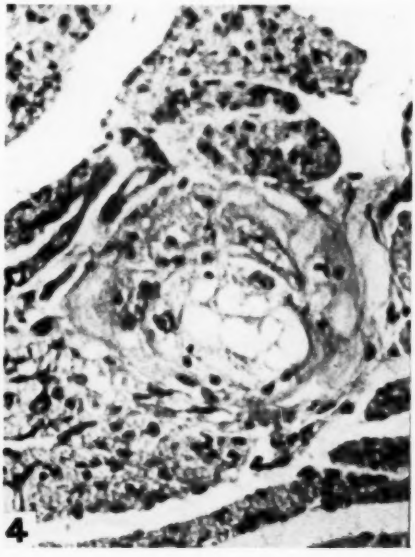
Fig. 2. Part of a nodule in the left ventricle. × 340.

Fig. 3. The edge of a subendocardial nodule in a papillary muscle of the left ventricle. × 280.

Fig. 4. A very small nodule in the wall of the left auricle. × 280.







Olsen and Cooper

Nodular Glycogenic Degeneration

OSTEO-ARTHRITIS DEFORMANS OF THE TEMPOROMANDIBULAR JOINT *

WILLIAM H. BAUER, M.D.

(From the Department of Oral Pathology, St. Louis University, School of Dentistry, St. Louis, Mo.)

The contradictory opinions concerning the etiology and nature of osteo-arthritis deformans are responsible for the use of varied terms for this clinical entity that may involve any joint. The opinions of some authors, who base their conclusions on clinical observation and consider metabolic or endocrine disturbances, infectious disorders or senility as etiological factors in this joint disease, cannot be substantiated. A clear concept of this problem can be attained only through microscopic investigation of joints actually involved by osteo-arthritis deformans. Such studies evidence the fact that only two groups of arthritis can be distinguished; namely, an infectious inflammatory type and a chronic, proliferative, noninfectious inflammatory type.

Osteo-arthritis deformans is a chronic, proliferative, noninfectious inflammation which gradually leads to progressive mutilation of the joint. The inflammation is due chiefly to altered and impaired function of the joint. Further, this disturbance of the joint may be caused by injury of any type which decreases the elasticity of the articular cartilage, the main function of which is the protection of the subchondral bone against abnormal and damaging stresses. In other words, the cartilage serves as a cushioning mechanism. There may be mechanical trauma involving the joint directly or indirectly. Osteo-arthritis deformans can occur in young individuals (juvenile arthritis, Lang [1]). It may also occur as the result of abnormally intensive use of the joint, as happens in certain occupations, or it may be produced by the wear and tear of advanced age. At any rate, the loss of the elasticity of the joint cartilage exposes the subchondral bone to constant mechanical injuries produced through impaired function. The bone marrow of the subchondral bone reacts to such prolonged traumata by inflammatory changes, resulting in osteoclas-

* Received for publication May 12, 1940.

tic resorption of the subchondral bony plate, and in proliferation of blood vessels and fibrous tissue into the damaged articular cartilage. This vascularization of the damaged cartilage, which is followed by ossification, gives rise to the development of marginal exostoses that may be covered with cartilage, or may be without cartilage, as in those cases where the cartilage is worn away. The marginal exostoses are the most characteristic feature of osteo-arthritis deformans.

The functional theory of the development of osteo-arthritis deformans was developed by Beneke,[2] Pommer [3] and Lang [1] after Nichols and Richardson [4] had given a very exact description of the microscopic findings of this joint disease. The functional theory was confirmed by the studies of Allison and Ghormley,[5] Parker, Keefer, Myers and Irwin,[6] and others.

Almost all human joints have been subjected to microscopic study in order to find an explanation for the structural changes and the etiology of osteo-arthritis deformans except the temporomandibular joint. This joint has an exceptional place among the other joints due to its unique anatomical configuration and its more complicated function. The temporo-mandibular joint is a two compartment joint because of its division into a menisco-temporal and a menisco-condylar joint by the disk. However, all articular movements of the joint occur simultaneously in both compartments. The movements consist of rotary motion, antero-posterior gliding, lateral motion and circumduction. Circumduction is a combination of all the other movements and is the correct and ideal masticatory motion. Both left and right temporomandibular joints function simultaneously, one influencing the other.

It is important to remember that parts of the temporomandibular joint articulation are not developed at the time of birth. The articular tubercle is developed in the child as the result of the special function that it must serve. Furthermore, the normal relationship between the condyle and the mandibular fossa is determined and maintained by the harmonic balance of the complement of teeth, particularly by normal occlusion of the lateral teeth. The loss of lateral teeth produces a change in the location of the condyle. With the loss of all teeth, this interarticular relationship will eventually become more and more discordant. The

condyle of a normally functioning temporomandibular joint, in the closed position, is located adjacent to the posterior articular plane of the articular tubercle. This is the starting point from which all articular movements begin. Subsequent to the loss of lateral teeth, or more particularly to the loss of all teeth, the condyle, in closed position, is displaced posteriorly and superiorly in the mandibular fossa. It is obvious that such a change in the starting position of the condyle will be a decisive factor in guiding the condyle along improper surfaces in abnormal and unharmonious movements. These considerations indicate that disturbances of the balance of the complement of teeth may bring about pathological changes in the temporomandibular joint due to disordered function. That the temporomandibular joint articulation performs so many complicated movements makes it most satisfactory for the purpose of studying the traumatic alterations of the articular cartilage and their consequences.

In 1932 I [7] published the results of a microscopic study of thirty-two temporomandibular joints obtained from cadavers ranging in age from 3 months to 68 years. These jaws varied from a full normal occlusion to complete edentulism. In most of them structural changes of the condyle, the disk, the articular tubercle and of the mandibular fossa were observed in varying degrees. These microscopic findings demonstrated that abnormal functional stresses, applied to the temporomandibular joint under certain anatomical or constitutional conditions, may lead to the characteristic changes of osteo-arthritis deformans (exostoses). Thus was made evident the importance of impaired function, *i.e.*, functional trauma or trauma of other origin, as the etiological factor in the development of osteo-arthritis deformans (Fig. 1). These findings were later confirmed by Steinhardt.[8] Roentgenographic studies of the diseases of the temporomandibular joint have been conducted by Goodfriend,[9] Riesner,[10] and others.

I have since conducted microscopic studies on the temporomandibular joints of five additional cadavers. The findings are of significance because there was opportunity to examine the patients before death. It was possible to compare the clinical histories with the microscopic findings and thereby prove that, due to the accommodation of this joint to pathological function, morphological alterations, even to the degree of a complete

destruction of the disk, were not associated with marked subjective symptoms.

Normal Structure of the Articular Cartilage and the Disk

In adults the articular surfaces of the condyle, the mandibular fossa and the articular tubercle are all normally covered by cartilage (Fig. 2), which consists of three distinct layers (Fig. 3). The innermost layer immediately adjacent to the subchondral bone is a zone of hyaline cartilage containing large basophilic cartilage cells. The second or middle layer is composed of a narrow strip of less basophilic cartilage cells arranged parallel to the surface of the bone. A dense accumulation of spindle-shaped cells separates the second layer from the third or outermost zone, which is wider and is made up of fibrocartilage, consisting of many fibers running parallel to the surface, and of a relatively few flat cells. This zone of fibrocartilage, resembling the cartilage of the clavicle and of the symphysis pubis, is covered by a very thin endothelium-like layer and, peripherally, there is a smooth transition into the stratum fibrosum of the periosteum and into the joint capsule (Fig. 2). This connection with the joint capsule is of great importance in solving the problem dealt with in this paper.

Relative to its cushioning function, the normal articular disk is made up of collagenous fibrous tissue arranged parallel to the surface of the condyle, and containing relatively few scattered chondroid cells grouped here and there. Elastic fibers are demonstrable between the collagenous fibers. Blood vessels are found in the central and peripheral portions of the disk. They assume a peculiar loop formation in the lateral or peripheral parts that are connected with the capsule (Fig. 2). This coil-like loop formation of the blood vessels indicates the compressibility of the disk; also its chondroid cells point to its mechanical function.

Changes in the Articular Cartilage

Pathological changes of the articular cartilage were found in almost all of the specimens that I examined, which included jaws of children with deciduous teeth, jaws of adults with a full com-

plement of teeth, and jaws of the toothless adult. The degree of alteration varied according to age and was much more intense in the cases where there had been a loss of lateral teeth or of all teeth.

In children the articular cartilage is strikingly different from that of adults. In children one does not find the calcified layer of cartilage or the subchondral bony plate. The trabeculae of the subchondral bone are arranged perpendicular to the articular surface of the condyle and the spaces of the bone marrow communicate directly with the articular cartilage, which is composed of fibrocartilage. This fibrocartilage consists of a layer of collagenous fibers which run radially from the surface of the subchondral bony trabeculae to the cartilage zone, where they turn tangentially and extend to the surface. This radially arranged layer contains relatively few cartilage cells.

The surface of the articular cartilage of the condyle, as well as that of the articular tubercle, of joints obtained from youths of 12, 17, 19 and 23 years of age shows a partially fringed appearance, whereas the inner portion is pierced by blood vessels which are accompanied by connective tissue advancing from the subchondral bone marrow. There are islands of bone surrounded by callus formation embedded in the cartilage.

Changes in the articular cartilage of the joints of older individuals are observed in the central areas of the articular surface and appear to be more severe. In addition to the development of fringes there are more or less extensive surface erosions. Associated with horizontal crevices of the superficial cartilage layer, it is frequently possible to observe vertical cracks starting on the surface and extending partly or even completely through the entire cartilage, and sometimes penetrating the subchondral bony plate. There are large horizontal fissures filled with blood which undermine the cartilage and separate it from the subchondral bone (Fig. 4). Furthermore, there may be complete destruction of the cartilage in localized areas, associated with an accumulation of broken down cartilage and callus-invested bone (Fig. 5). Occasionally this detritus forms a cyst which occupies the entire thickness of the cartilage layer. Vertical fibrillation of the cartilage can be seen extending through the calcified cartilage and the subchondral bony plate into the bone marrow. Superficial

cartilage cells are flattened due to pressure, whereas other cells show degenerative changes of all degrees to complete vacuolar degeneration. In some cases these alterations involve the basal calcified layer, thus destroying the last protective barrier of the subchondral bone (Fig. 6).

These marked changes in the cartilage are associated with traumata of various kinds. The alterations of the cartilage, which involve the cartilage cells as well as their ground substance, are, according to Pommer,[3] due particularly to edema, which is the result of the chronic traumata caused by impaired function. Further evidence of this concept has recently been presented in the studies of Callender and Kelser.[11] These authors described the presence of "blister" formations on the surface of the articular cartilage of human and animal joints and spoke of edematous or swollen cartilage. These "blisters" rupture and discharge a fluid into the joint cavity. The surface fringes of cartilage, common to this disease, are the remnants of these ruptured "blisters." Moreover, occasionally these fringes may result also from simple damaging stresses applied to the cartilage, with the same result as is seen in nicking a razor strop with a razor. In some of the cases of osteo-arthritis deformans which I studied, I found complete destruction of the cartilage layer with complete denudation of the subchondral bone, as has been described by Parker and co-workers.[6] Occasionally, in regions where injury has been sustained over a long period of time, a diffuse calcification of the altered cartilage tissue occurs (Fig. 7). Such calcified areas, extending in some cases to the surface, induce ossification so that they become entrapped in bone. The surface of the articular cartilage assumes a hyaline appearance early. In a very advanced case of osteo-arthritis deformans of the temporomandibular joint in which the disk was destroyed, a portion of the condylar cartilage was necrotic.

Regenerative processes in cartilage can be seen as well as the degenerative changes described above. The most important finding in the damaged and inelastic cartilage is the vascularization which proceeds from the subchondral bone marrow. Vascularization (Fig. 8) is followed by ossification of the articular cartilage which is the phenomenon by which the characteristic exostoses are formed (Randwülste). The traumatic changes of the carti-

lage and the vascularization of cartilage from the opened marrow spaces can be observed in all articular planes, but more particularly in the peripheral regions. This striking finding is readily explainable because the cartilage at its periphery is intimately connected with the tissue of the joint capsule, as well as with that of the periosteum and therefore it is particularly exposed to tearing stresses that tend to detach it from the subchondral bone if the stresses exerted on the capsular tissue exceed the biologic limits.

Changes in the Subchondral Bone

In the adult temporomandibular joints the borderline between the subchondral bony plate and the calcified articular cartilage is very irregular since there are areas of cartilage deeply embraced in the bone trabeculae. These findings point to an irregularity of ossification which can be explained by the fact that at the time of ossification, during youth, abnormal functional stresses disturb and inhibit uniform ossification.

Clefts, filled with blood, are sometimes observed perforating the subchondral bony plate and entering bone marrow spaces. Even in the absence of any changes of the covering cartilage this process is seen. In those cases where lesions of the cartilage had penetrated to the subchondral bone, resorption of the external surface of the subchondral bone by osteoclasts is observed and is also sometimes accompanied by osteoclastic resorption proceeding from the bone marrow spaces outward. Such bone marrow, however, is not of the normal fatty type but has been changed into fibrous tissue due to its reaction to irritative stresses (Fig. 6). In advanced lesions there are observed fractures of the subchondral bony plate with openings into the bone marrow despite the fact that in some cases the covering cartilage was partially maintained. I was able neither to observe any marked thickening of the original subchondral bone due to traumatic changes of the cartilage nor to see any necrosis of bone following fragmentation of the cartilage, as stated by Parker and co-workers.[6]

The fibrous bone marrow contains dilated blood vessels and it occasionally communicates with the altered cartilage (Figs. 6 and 9). Islands of bone produced by osteoclastic resorption along

with hemorrhages embedded in callus are found scattered in both the cartilage and the bone marrow spaces.

In addition to the degenerative changes of the cartilage, callus formation of young cartilage in connection with the traumatic interruption of the subchondral bony plate is present. This cartilaginous callus passes through the resorbed bony border and enters the marrow spaces (Figs. 10 and 11). In such an area I found a large cyst lined by connective tissue and partially surrounded by young cartilage cells with an island of cartilage cells in the periphery.

It is rational to assume that the islands of cartilage located in traumatically opened bone marrow spaces were displaced forcibly into them. However, the finding, in cases of extensive lesions, of islands of cartilage in distant marrow spaces containing normal fatty marrow has evoked many different explanations. According to Pommer [3] and Lang,[1] these small, round islands of young cartilage cells, surrounded by very thin and small spindle-shaped cells (endothelial cells), which are found in unchanged bone marrow, were carried there by the blood or lymph as emboli. Of a contrary opinion were Parker and his co-workers [6] who contended that these cartilage cells, at some distance from the joint line, were produced by the endosteum of the surrounding bone trabeculae or from the connective tissue of the marrow by means of metaplasia. Since it is a well established and generally accepted fact that cartilage is a product of grinding or rubbing stresses, I cannot bring myself to believe that bone marrow located so far away from the area of traumatic irritation could be exposed to such cartilage-inciting stresses, particularly since the normal fatty marrow remains unchanged.

Exostoses

While the exostoses are generally believed to be the most significant finding in osteo-arthritis deformans, there is as yet no uniform opinion concerning their origin. According to Nichols and Richardson [4] these characteristic bony protuberances are the result of perichondral or subchondral proliferation and develop by ossification of cartilaginous overgrowths. However, Pommer [3] and Lang [1] proved, by their microscopic studies, that the exostoses arise from the subchondral bone marrow after vascularize-

tion of the damaged and inelastic articular cartilage. Despite the fact that this explanation, based on careful microscopic study, was confirmed by Erdheim,[12] Burckhardt [13] and myself [7] and more recently by Callender and Kelser,[11] there are other authors who still give a different explanation. Walter Bauer [14] contended that this overgrowth originates as a result of the proliferation of the marginal area of the cartilage which later is transformed into true bone. Parker and co-workers [6] believed that "flattening of the surface which is in contact with the opposing joint surface causes the edge of the bone to project outward as a shelf, and, depending on weight, pressure and position, the projection may curl or bend over at the edge."

In my opinion, the microscopic findings of this study give clear evidence that true exostoses originate from the subchondral bone marrow that first renders vascular and then ossifies the damaged cartilage. These exostoses develop not only in the peripheral cartilaginous area in which they overlap the bone, so that they take the appearance of a mushroom, but they also occur in the central portion of the articular plane, thus enlarging the condyle in its longitudinal axis. I could find no evidence to support the theory that the bone is forced outward by a central pressure. It is difficult to believe that such well calcified bone as the subchondral bone could be compressed centrally with curling and bulging of its periphery. In 1932 I [7] described and illustrated the marked longitudinal enlargement of the condyle, clearly proving that the growth therein is uniform and cannot be accounted for by pressure activity. Such exostoses as shown in Figure 11 have highly polished surfaces and are partly covered by areas of necrotic articular disk. The periosteum does not directly participate in the formation of the marginal exostoses. However, osteophytes from periosteal bone formation may occasionally unite with these exostoses.

Changes in the Disk

Such pathological changes of the temporomandibular joint as have been described above must exert a damaging influence on the articular disk. The disks of the temporomandibular joints that I have studied reveal the same alterations as the articular

cartilage. Cracks and fissures can be seen which are followed later by hyalinization of the disk tissue. One disk in particular shows extensive calcification of its dystrophic tissue while the disks of the joints with exostoses are either necrotic in those areas adjacent to the exostoses or are partially destroyed so that the joint becomes practically diskless.

Summary

Forty-two human temporomandibular joints from individuals ranging from 3 months to 81 years of age were studied microscopically. Death had resulted from various diseases.

1. Most of the temporomandibular joints showed traumatic changes of varying degree. These changes primarily involved the cartilage of the condyle, the articular tubercle, the mandibular fossa and finally the disk structure.

2. The lesions were produced by impaired function through disturbed balance of the involved joint. The disturbed balance was due either to the loss of lateral teeth, loss of all teeth or to an external injury. All of these disturbances indirectly caused decrease or loss of elasticity of the cartilage, whose main function is the protection of the subchondral bone against abnormal functional stresses. This less elastic cartilaginous layer showed cracks, fissures and fringes. The cartilage cells underwent various types of degenerative changes such as fatty and mucoid degeneration, while on the other hand an overgrowth of the cartilage cells was observed. Also, dystrophic calcification of the cartilage, followed by ossification, was an occasional finding.

3. Involvement of the articular cartilage was occasionally followed by vascularization of the cartilage proceeding from the subchondral marrow after the subchondral bony plate had been resorbed. The normal fatty marrow of these opened spaces was transformed into a fibrous or gelatinous tissue containing dilated blood vessels, hemorrhages, areas of callus formation, accumulations of detritus and cartilage islands.

4. Vascularization and ossification of the cartilage layer produced exostoses that consisted of laminated bony trabeculae and contained cartilage cells which had escaped resorption. Marginal exostoses of the condyle occurred in areas where the cartilage was exposed to unfavorable stresses, and particularly, because of

its connection with the capsule tissue and the disk, when the equilibrium of the joint was disturbed. The periosteum did not actively participate in the formation of exostoses but deposits of incidentally formed periosteal bone may be united to the true exostoses. Furthermore, exostoses were formed in the central parts of the condyle, thus producing deformation in the longitudinal axis.

5. The disk showed all degenerative changes up to complete destruction, depending on the severity of the lesion.

6. Functional traumata of the cartilage of the temporomandibular joint promote the development of osteo-arthritis deformans.

7. Osteo-arthritis deformans is a chronic noninfectious proliferative inflammation.

REFERENCES

1. Lang, F. J. Arthritis deformans und Spondylitis deformans. Handbuch der speziellen pathologischen Anatomie und Histologie. Arranged by O. Lubarsch and F. Henke. J. Springer, Berlin, 1934, **IX/2**, 252–376.

2. Beneke, R. Zur Lehre von der Spondylitis deformans. Beitr. z. wissensch. Med. Festschr. . . . d. Versamml. deutsch. Naturf. u. Aerzte. . . . , Brnschwg., 1897, 109–131.

3. Pommer, G. Über die mikroskopischen Kennzeichen und die Entstehungsbedingungen der Arthritis deformans (nebst neuen Beiträgen zur Kenntnis der Knorpelknötchen). *Virchows Arch. f. path. Anat.*, 1927, **263**, 434–514.

4. Nichols, Edward H., and Richardson, Frank L. Arthritis deformans. *J. M. Research*, 1909, **21**, 149–221.

5. Allison, Nathaniel, and Ghormley, Ralph K. Diagnosis in Joint Disease. A Clinical and Pathological Study of Arthritis. William Wood and Company, New York, 1931.

6. Parker, Frederic, Jr., Keefer, Chester S., Myers, Walter K., and Irwin, Ralph L. Histologic changes in the knee joint with advancing age; relation to degenerative arthritis. *Arch. Path.*, 1934, **17**, 516–532.

7. Bauer, William H. Anatomische und mikroskopische Untersuchungen über das Kiefergelenk mit besonderer Berücksichtigung der Veränderungen bei Osteo-Arthritis deformans. *Ztschr. f. Stomatol.*, 1932, **30**, 1136; 1279.

8. Steinhardt, Gerhard. Zur Pathologie des Kiefergelenkes. *Paradentium*, 1932, **4**, No. 4, p. 111; No. 6, p. 153.

9. Goodfriend, David J. Abnormalities of the mandibular articulation. *J. Am. Dent. A.*, 1934, **21**, 204–218.

10. Riesner, Sidney E. Temporomandibular reactions to occlusal anomalies. *J. Am. Dent. A.*, 1938, **25**, 1938–1953.

11. Callender, G. R., and Kelser, R. A. Degenerative arthritis. A comparison of the pathological changes in man and equines. *Am. J. Path.*, 1938, **14**, 253–272.

12. Erdheim, J. Die Lebensvorgänge in normalen Knorpel und seine Wucherung bei Akromegalie. In: Pathologie und Klinik in Einzeldarstellungen, III. Julius Springer, Berlin & Vienna, 1931.

13. Burckhardt, Hans. Arthritis deformans und chronische Gelenkkrankheiten. *Neue Deutsche Chir.*, 1932, **52**, 464 pp.

14. Bauer, Walter. Studies pertaining to the origin and nature of hypertrophic arthritis. *Tr. & Stud., Coll. Physicians, Philadelphia*, 1939, **7**, 1–20.

DESCRIPTION OF PLATES

PLATE 28

FIG. 1. Pumice-like surface of a malformed condyle.

FIG. 2. A section from a normal temporomandibular joint, in close bite, of a woman 28 years old. Note the connection of the marginal articular cartilage with the tissue of the capsule and disk. × 4.

FIG. 3. Normal cartilage of condyle separated by joint space from disk. × 96.